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USE OF WATER BY CULTIVATED PLANTS IN THE FIELD.

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INTRODUCTION.

THE problem of the use of water by cultivated plants during the period of their growth is of great scientific interest. It is certainly of prime interest for the farmers of a dry region where to secure a yield everything depends in most cases upon the problem of moisture.

When studying the problem of the use of water by cultivated plants under field conditions the importance of extremely unstable and widely varying meteorological factors is evident. The fluctuations are especially large and irregular with respect to moisture.

To determine to a certain degree the importance of this factor of variation in weather during the growth period it is necessary to carry out observations and experiments on the determination of the moisture consumed by cultivated plants over several years, to make these observations at different places in a dry region and to control the conduct of plants at these places during the same year.

Both methods were used by us in the investigational work of the Experimental Station. Observations concerning the use of moisture by cultivated plants in the field were started at the Experimental Station in 1924. They were carried on for a considerably larger range of plants in 1925 and 1926, years differing greatly in their meteorological conditions. In the spring of 1926 we succeeded in securing the cooperation of some district experimental stations of the dry region. The result is that we are in possession of a mass of data for 1926 gathered at different stations and for different plants on the basis of coordinate methods.

HISTORY OF THE PROBLEM.

There exists a small amount of literature on the problem of the use of water by cultivated plants in the field describing methods similar to those developed in our studies. In a preliminary manner this problem was undertaken by us fifteen years ago at the Besentchuk Experimental Station, when we determined the quantity of water used by the plant from the final and initial (during harvest and sowing time) water supply

stored in the soil and the quantity of water added by precipitation during the growth period of various plants. The quantity of water used was divided by the dry weight of yield. We thus obtained the so-called "transpiration coefficient" from the field observations.

The same method was used by G. E. Mordovsky in his study concerning the rate of the use of water by different plants. This study contains a volumė of observations made in 1925 for winter rye, sunflower, haricot, mustard, potatoes and corn at the Vosnesensk experimental field. The total quantity of water used by winter rye during its growth period was 304·6 millimetres.

The second study on the same problem was published by V. I. Efimov in the *Report on the work conducted by Krasnokutsk Experimental Station during* 1925 *and in connection with previous years.*

The observations were made for a range of plants and the most interesting of them have given the following figures:

Plants	Transpiration coefficient
Wheat Beloturka	399
Wheat Russak	358
Millet	233
Maize for green food	433
Sudan grass	872
Alfalfa in the beginning of blooming	1175

A partial discussion of the problem, for maize and spring wheat only, was given by A. F. Lebedev.

A large mass of observations with respect to this problem was published in 1927 by V. I. Ilyin in two issues of the magazine *Experimental Agronomy of the South-East.*

Method of Investigation.

The whole study of the problem of moisture consumption by cultivated plants in the field was conducted on the basis of a uniform method.

When calculating the quantity of moisture used by the plant during the whole period of its growth, the total amount of rainfall between the subsequent determinations of soil moisture and simultaneous gathering of samples of plants was taken into consideration.

Observations on soil moisture and the growth of the dry weight of the plant were regularly made on the 5th, 15th and 25th of each month. During the second half of the plant's growth period observations were made every five days. They give a more accurate determination of the accumulation of dry weight and the consumption of water by the plant. Absolute quantity of consumed moisture was determined by using the

percentage of soil moisture and its apparent specific gravity in different horizons as bases from which to compute departures. Determinations of moisture were made at intervals of 10 centimetres to the depth of 1 metre and reckoned in tables for the whole layer 1 metre deep.

The quantity of water consumed was calculated in terms of the dry yield of the plant.

METEOROLOGICAL CONDITIONS OF THE GROWING SEASON OF 1926.

For most of the experimental stations where this experiment was conducted the growing season of 1926 can be stated as moist and moderately warm. Spring was almost everywhere late and adequately moist, and the sowing of spring crops was carried on in favourable conditions. The summer was moderately warm with adequate rainfall. It may be noted that the temperature of the air acted as a limiting factor for the growth of intertilled crops, postponing their ripening and prolonging their period of growth.

To characterise the growth period of spring wheat in 1926, the following figures of rainfall during the period of its growth are shown for different localities of the experiment:

Experimental stations	Rainfall in millimetres
Saratov	170
Balashov	135
Krasnokutsk	143
Kostychev	153
Kamyshin	96

In all these localities the largest quantity of water and the heaviest rains fell in the beginning of the spring wheat's growth period. But there was also everywhere a small but sufficient amount of rain towards the time of its ripening.

In any case with respect to rainfall the year must be considered as a favourable one. As concerns temperature and rainfall the year was especially favourable for oats and winter rye which produced in this year a better yield than the average.

EXPERIMENTAL.

When examining the resulting figures, tables were made out for each separate plant at each stage in the experiment, giving all data in figures which characterise the use of water from the soil, the growth of the dry weight of the plant, and the rainfall during the time between successive observations.

Winter rye. Determination of the use of water by winter rye and the increase of its dry weight was made at the Saratov, Balashov, Krasnokutsk and Kamyshin Experimental Stations possessing very different soils.

Table I. *Weight of water evaporated from the soil cropped to winter rye up to the day of sampling (in tons per hectare).*

	Saratov Experimental Station				Balashov Experimental Station	Krasnokutsk Experimental Station	Kamyshin Experimental Station
Date of observations	Dark chestnut soil	Sand soil	Southern dark coloured soil	Alkali soil	Fertile dark coloured soil	Chestnut soil	Light chestnut soil
May 5	227	176	252	13	231	127	140
„ 15	367	533	443	228	407	513	428
„ 25	959	1087	1399	695	1179	873	750
June 1	1123	1339	1450	1047	1404	1233	1079
„ 5	1158	1490	2143	1173	1490	1265	1303
„ 15	1363	1654	2496	1438	1786	1479	1417
„ 25	1759	1849	2665	1507	2166	1885	1963
July 5	2306	2115	2776	1693	2478	2026	2075
„ 15	2323	2132	2860	1878	3403	2120	2204

Table II. *Transpiration coefficient of winter rye by decades and during growth.*

	Saratov Experimental Station				Balashov Experimental Station	Krasnokutsk Experimental Station	Kamyshin Experimental Station
Date of observations	Dark chestnut soil	Sand soil	Southern dark coloured soil	Alkali soil	Fertile dark coloured soil	Chestnut soil	Light chestnut soil
May 5	87	—	97	7	642	1297	121
„ 15	119	664	120	74	415	268	227
„ 25	135	—	269	108	—	229	—
June 1	88	394	218	175	385	154	174
„ 5	102	385	222	175	448	153	157
„ 15	108	414	130	128	284	213	178
„ 25	138	430	190	142	399	181	152
July 5	166	529	—	—	256	227	247
„ 15	215	418	178	200	326	264	234

Summarising the data available on winter rye, the following results may be noted.

Up to the attainment of the earing stage, winter rye transpires on an average 54 per cent. of total moisture used by it from the soil during its growth period. A figure near to it was obtained for Saratov in 1924, an extremely dry year, and a smaller figure (50 per cent.) in the moist year 1925. An increased consumption of water continues during the stages of

earing and blooming, and by the time of filling winter rye has used almost 75 per cent. of the total moisture required by it.

Winter rye shows a rapid increase in dry weight in the beginning of its spring growth. In the comparatively moist year 1926 winter rye had accumulated about 58 per cent. of its resulting yield by the stage of earing. Almost the same quantity was accumulated by it under Saratov conditions in the moist year 1925, and a considerably larger quantity in the dry year 1924 (80 per cent.).

The transpiration coefficient of winter rye varies comparatively little, and for 1926 its mean value was 265. The experiments conducted at Saratov in the moist year 1925 have given 242 as transpiration coefficient and in the dry year 1924, 202.

Assuming that winter rye has used the total quantity of water supplied by precipitation during the period of its growth, it will appear that 63·4 per cent. of its total requirement in water was supplied in 1926 by precipitation during the period of growth and only 36·6 per cent. was used from the spring water supply stored in the soil. In this sense 1926 differs considerably from the dry year 1924 when the largest portion of total moisture needed was used from the soil and only 30 per cent. was supplied by precipitation during the growth period. In 1925 the water used was also largely represented by that stored in the soil. The situation of 1926 is due to a more favourable distribution of rainfall during the period of the rye's growth, as the portion of water added by it was larger during the first half of the winter rye's growth period.

Spring wheat. A collective experiment concerning spring wheat was conducted at the same experimental stations as in the case of winter rye. Moreover, the workers of Volsk Agricultural College have taken part in this experiment and in an experiment with respect to the determination of moisture used by spring wheat that was conducted on irrigated and non-irrigated sections of a field at the Kostychev Experimental Station.

The data available on the problem of the use of water by spring wheat during the period of its growth in the field, together with the experiments conducted at the Saratov Experimental Station in 1924 and 1925, lead to the following general results.

In spite of the fact that up to the stage of tillering, spring wheat matures a comparatively small portion of its dry yield, it transpires during this period more than one-half of the total quantity of water required by it, using it during this period without effect. A considerably increased use of water also goes on during the two following decades

until the beginning of blooming before which time spring wheat uses on the average about 78·9 per cent. of total moisture needed.

Table III. *Weight of water evaporated from the soil cropped to spring wheat up to the day of gathering samples (in tons per hectare).*

Date of observations	Saratov Experimental Station: Dark chestnut soil	Saratov Experimental Station: Alkali soil	Balashov Experimental Station: Fertile dark coloured soil	Krasnokutsk Experimental Station: Chestnut soil	Volsk Agricultural College: Dark coloured soil	Kamyshin Experimental Station: Light chestnut soil	Kostychev Experimental Station, Brown soil: Non-irrigated	Kostychev Experimental Station, Brown soil: Irrigated
May 15	—	504	136	—	—	—	—	—
„ 25	518	995	908	416	—	480	181	717
June 5	1022	1128	1258	829	—	897	931	717
„ 15	1338	1367	1535	1011	142	1123	1410	2447
„ 25	1683	1562	1861	1523	516	1949	1790	3159
July 5	1962	1692	2256	1611	881	2117	2078	3721
„ 15	1978	1712	2465	1832	1360	2267	2328	5083
„ 25	—	1733	2490	2305	2069	2524	2359	5704
„ 30	2223	2074	—	—	—	—	2359	—
August 5	2223	2074	2200	2479	2140	—	—	6193
„ 10	—	—	2203	—	—	—	—	6240

Table IV. *Transpiration coefficient of spring wheat by decades and during growth.*

Date of observations	Saratov Experimental Station: Dark chestnut soil	Saratov Experimental Station: Alkali soil	Balashov Experimental Station: Fertile dark coloured soil	Krasnokutsk Experimental Station: Chestnut soil	Volsk Agricultural College: Dark coloured soil	Kamyshin Experimental Station: Light chestnut soil	Kostychev Experimental Station, Brown soil: Non-irrigated	Kostychev Experimental Station, Brown soil: Irrigated
May 20	—	—	—	—	—	3863	—	—
„ 25	—	—	—	5940	—	—	—	—
June 5	2044	3138	—	—	—	1900	2908	2866
„ 15	1216	1119	1150	—	508	1040	1900	—
„ 25	748	447	865	—	430	815	1201	2411
July 5	548	412	778	—	400	628	808	1343
„ 15	452	300	685	—	371	—	817	1193
„ 25	—	419	553	—	401	700	678	1546
„ 30	436	376	—	—	—	—	643	—
August 5	—	—	492	443	304	—	—	1337

From all data furnished during three years of observations it is evident that spring wheat uses during the whole period of growth about 2400 tons of water per hectare. This quantity varies in different years and on different soils from 2000 to 3185 tons. It coincides exactly with the average quantity of water used by winter wheat during its spring and summer growth. It seems that in the conditions of a dry region, winter and spring crops use during their growth all moisture that can be stored in the soil during spring. In the years with high rainfall the total weight of moisture used by wheat during its growth period is larger than that

used in dry years. In this sense different soils differ from each other to a rather small extent.

The transpiration coefficient of spring wheat varies at different places to a considerable extent. It is considerably higher for the southern experimental stations than for the northern ones. It is also much higher for wheat on irrigated sections of the field. Owing to the smaller dry weight of spring wheat as compared with winter rye, the transpiration coefficient of spring wheat is for any given year considerably higher than that of winter rye. It also varies more with different soils and in different years.

The rapid growth of the dry weight of spring wheat goes on in the period between the time of tillering and the time of blooming, when the greater portion of the weight is accumulated. The accumulation of dry weight also goes on rapidly up to the attainment of the crop's "milk" stage. In the beginning of its growth, spring wheat increases its dry weight very slowly.

When distinguishing between water used by spring wheat from the initial water supply stored in the soil, and water supplied to it by rains during the period of growth, it appeared that in the moist year 1926 the largest portion of total moisture needed was supplied by precipitation during the growth period (63·6 per cent. average). In this sense Kamyshin occupies rather a different position. Here rainfall has supplied only 38·7 per cent. of spring wheat's total requirement in water: that is approximately as much as was noted for Saratov in the dry year 1924. From the data available it can be established as a rule that in a dry region spring wheat uses about 70 per cent. of total water needed from the initial water supply stored in the soil. In a moist year from 55 to 65 per cent. of the total water requirement is supplied by precipitation during the growth period and the rest is used from the soil. The average rainfall during the growth period of wheat was for all stations 141 millimetres.

In the experiment conducted at the Kostychev Experimental Station on the irrigation of spring wheat, it appeared that only 24·2 per cent. of wheat's total requirement in water was supplied by precipitation and the remaining 75·8 per cent. was used from the soil. At the same time on the non-irrigated sections of a field at the Kostychev Experimental Station rainfall has supplied about 65 per cent. of total water needed and only 35 per cent. was used from water supply stored in the soil.

Oats. As concerns soil moisture and rainfall, oats in Balashov were like wheat, differing from it to a rather small extent in the character of growth and in the use of water from soil.

Corn. Observations concerning the growth of corn were made in 1926

at the Saratov, Balashov, Krasnokutsk, Kostychev and Kamyshin Experimental Stations.

Table V. *Weight of water evaporated from the soil cropped to corn up to the day of gathering samples (in tons per hectare).*

Date of observations	Saratov Experimental Station Dark chestnut soil	Balashov Experimental Station Fertile dark coloured soil	Krasnokutsk Experimental Station Chestnut soil	Kamyshin Experimental Station Light chestnut soil	Kostychev Experimental Station Brown soil non-irrigated
May 25	50	504	—	—	—
June 5	—	570	170	—	—
„ 15	538	620	373	310	323
„ 25	787	916	588	310	752
July 5	944	1065	909	562	920
„ 15	1045	1679	971	803	—
„ 25	1313	1836	1297	969	1710
Aug. 5	1617	1955	1576	1108	1732
„ 15	2178	2207	1599	1630	1841
„ 25	2378	—	1796	—	2066
Sept. 5	—	2770	2029	1766	—
„ 15	2582	—	2229	1852	2137
„ 20	—	—	—	1888	—
„ 25	2770	—	2332	—	—

Table VI. *Transpiration coefficient of corn by decades and during growth.*

Date of observations	Saratov Experimental Station Dark chestnut soil	Balashov Experimental Station Fertile dark coloured soil	Krasnokutsk Experimental Station Chestnut soil	Kamyshin Experimental Station Light chestnut soil	Kostychev Experimental Station Brown soil non-irrigated
June 5	—	—	4988	—	—
„ 15	29860	31045	2624	—	11521
„ 25	13569	4584	3923	—	3760
July 5	1861	1522	1245	—	837
„ 15	908	1298	1234	—	—
„ 25	469	365	882	—	820
Aug. 5	337	337	637	—	712
„ 15	—	276	472	—	375
„ 25	301	—	535	—	578
Sept. 5	—	160	424	—	—
„ 15	276	—	252	—	466
„ 25	276	—	193	430	—

As a result of the observations made in 1926 at different experimental stations of Nijnee Povoljie and of the data gathered by means of observations on corn in 1924 and 1925 at the Saratov Experimental Station the following summary can be established.

Dividing the growth period of corn into four equal parts the use of water from soil goes on more or less equally during each month. But during blooming and the "milk" stage there is an increased use of water.

During the same period there goes on also the rapid growth of corn when it matures approximately one-half of its total dry yield.

The transpiration coefficient of corn for different parts of the region varies comparatively little. It is comparatively low and is considerably higher for the southern than for the northern parts. There is no noticeable difference in the value of the transpiration coefficient for years differing in dryness. For example, during the last three years (of which 1924 was very dry), the transpiration coefficient of corn at the Saratov Experimental Station was successively 443, 290 and 276.

A considerable portion of the water required by corn is supplied by precipitation during the period of growth, and the rest is used by it from the initial water supply stored in the soil. Even in the dry 1924 rainfall has supplied 65 per cent. of the total water needed, and in a moist year the portion supplied by it increases considerably. The same was noted in 1926 at different stations of the region.

Millet. Observations with respect to millet were made at the following places: Anuchino, Volsk, Balashov, Saratov, Krasnokutsk and Kamyshin.

The transpiration coefficient of millet during growth has fluctuated from 241 at the Kamyshin to 400 at the Balashov Experimental Station.

The rapid growth of millet begins during the second third of its total growth period. During the first third it commonly produces only a small portion of its dry yield.

The loss of moisture from the soil goes on more evenly, but still the second third of the growth period has in this respect the greatest importance. The detailed observations establish the fact of the largest consumption of water during the stage of blooming.

The transpiration coefficient of millet is relatively very low, and in dry years or in the conditions of a dry and more warm region it is noticeably lower than otherwise.

In all cases a considerable portion of the moisture needed is supplied by rainfall during the growth period and the rest is used from the water supply stored in the soil prior to sowing. This is due to the long growth period of millet and to its comparatively slow development during the first half of its growth.

Sunflower. A collective experiment with sunflowers was conducted in 1926 at the Saratov, Volsk, Balashov and Krasnokutsk Experimental Stations.

In the beginning of its growth period the sunflower grows very slowly and uses during this time the largest portion of water needed. The rapid growth of the sunflower begins from the time of the development of the

"flower" bud, and by the time of blooming the sunflower accumulates about two-thirds of its dry weight.

Owing to its long growth period, the sunflower uses a considerable quantity of moisture. This explains also the fact that about 66 per cent. of total moisture needed is supplied by rainfall during the period of growth and only an inconsiderable portion is used from the soil.

Only two leguminous plants were investigated: lence at Saratov and Balashov and cicer at the Saratov, Krasnokutsk and Kostychev Experimental Stations.

As concerns forage plants observations on the use of water and the accumulation of dry weight were made with respect to Sudan grass (at all four experimental stations).

Like all other annuals Sudan grass uses during the first stage of its growth considerably more moisture than during the later stages. Therefore more moisture is used during the first than during the second cutting if there are two in all, or almost the same quantity if there are three cuttings.

The transpiration coefficient of Sudan grass is comparatively low. It increases considerably in a dry year, as shown by the figures for Saratov in 1924, and it decreases greatly in the moist years.

The limited growth of Sudan grass in 1926 was due, doubtless, to the low temperature.

The observations on sugar beet were made in 1926 at the two following places: at the Saratov and Balashov Experimental Stations.

In 1926, too, the loss of water by "black fallow" was studied at the Saratov, Balashov and Krasnokutsk Experimental Stations. Determinations were made every ten days during the period of fallowing. Fallow soil loses during summer a considerable quantity of water, approximately 2000 tons per hectare, that is, a little less than some cultivated plants.

Under arid conditions the soil is unable to store until sowing time water supplied by precipitation during summer. In the best cases it acts only as a reservoir of water stored in the soil during autumn, winter and early spring.

General Discussion of Results.

During the growing season of 1926 observation was made at six experimental stations of Nijnee Povoljie on 39 cases of the use of water by plants and fallow during the period of growth and the season of fallowing up to the sowing of winter crops. The weather of this year

may fairly be described as moist, but there was, of course, considerable variation in the rainfall at the different experimental centres. This has, without doubt, a great importance for the estimate of the results of observations in the future.

On the other hand, it is necessary to keep in view the facts that the observations stated above were made at different experimental stations, and although on the same plants, on very different soils. The last fact to remember is that the study was conducted on the basis of uniform methods, but by different persons.

In spite of all these considerations we think it worth while to use these data for a series of deductions which seem to us very interesting. It is also possible to use for the same purpose the data bearing on this problem collected at the Saratov Experimental Station in 1924 and 1925. In such a way, we are in possession of 80 cases of observations on the use of water by plants, and are therefore furnished with a large mass of data.

1. *The quantity of water used by the plant during its growth period.* In considering in the above tables the quantity of water used by the plants at different experimental stations, the following facts can be established. In spite of the soil differences the quantity of water required by spring wheat to mature its yield has varied in 1926 from 2074 to 2524 tons per hectare, and the mean was about 2290 tons. This mean for winter rye was 2340 tons, for millet 2340, for corn 2380, for cicer 2255, for beet 2315 and for sunflower 3047. So with the exception of sunflower very close figures for the use of water were obtained with all the experimental plants. One might even say that the figure is constant and that only sunflower has given a rather higher figure. The figures calculated for the dry year 1924 were a little smaller and the figures for the moist year 1925 considerably larger.

In the case of spring wheat, taking into account the data of 1924 and 1925, the average quantity of water required by spring wheat will be for all eleven observations 2347 tons per hectare.

These figures show that at different experimental stations spring wheat has needed on the average during this period 235 millimetres of water for the production of a mature crop. One portion of this quantity represents water supplied by autumn and winter precipitation and stored in the soil prior to sowing, the other was supplied by spring and summer rainfall during the period of growth.

In an investigation similar to ours carried out in the dry region of the U.S.A., data are given showing the quantity of water used by spring

wheat during growth at different experimental stations and in different years[1].

The data on the use of water are shown here for 96 cases. In these years good yields were obtained when the quantity of water used was large, and in very dry years, when little water was used, the yields were small. The average quantity of water used by spring wheat calculated by us on the basis of these data is 250 millimetres, a quantity extremely near to our average quantity of the use of water by spring wheat.

We are not speaking, at the present time, about the resulting yield which certainly was different in our and the American observations. From a large mass of observations Cole and Matthews draw the conclusion that a direct correlation exists between the quantity of water used by wheat and the resulting yield, and that in most cases the large quantity of water used is the deciding factor for the production of a better yield. They establish further that even for the production of an insignificant yield the use of not less than 100 millimetres of water from the soil is necessary at the northern experimental stations in a climate similar to ours and not less than 250 millimetres at the more southern ones.

A different situation results if we subtract the quantity of water supplied by rains during the period of the wheat's growth from the total water used, thus determining the quantity of water used by wheat from the initial water supply stored in the soil. The data obtained in our experiments of 1926 show precisely that at the southern experimental stations, where precipitation during the growth period of wheat was smaller, a considerably larger quantity of water was used from the soil and *vice versa*. The fluctuations were here from 500 to 1500 tons per hectare. Or, in other words, from 50 to 150 millimetres of water were used from the soil, with an average of 85 millimetres. The same figure for wheat calculated for all years of our observations has given 99 millimetres, and the average figure for all early crops (wheat, oats and barley) was in all cases 110 millimetres.

The approximate quantity of water evaporated from the soil during the growth of early crops and especially of spring wheat is thus about 235 millimetres. The portion of this quantity which was used from water stored in the soil is on the average 110 millimetres, and the portion supplied by precipitation during the period of growth, 125 millimetres.

A comparatively small number of observations on other plants does

[1] John S. Cole and O. R. Matthews, "Use of water by Spring Wheat on the Great Plains," *U.S. Department of Agriculture, Bul.* 1004 (1923).

not enable us to draw similar conclusions for them with any certainty. However, the following information can be supplied as data for future studies. The use of water from the soil was for winter crops (rye and wheat) 113 millimetres, that for beans (lence, peas, cicer) 100 millimetres, that for millet, corn and sorghum 76 millimetres, that for sunflower 98 millimetres. The longer the plant is in the field, the larger is the portion of its total water requirement which is supplied by rainfall. This statement is especially confirmed by the data for potatoes, beet and pumpkin.

2. The transpiration coefficient of different plants is either fairly constant at different experimental stations or varies considerably. It was found to be very constant for winter rye and corn, less constant for sunflower and millet and it varied considerably for spring wheat and beans.

In considering the data on all plants furnished by the Saratov Experimental Station during the period of three successive years of observations (1924, 1925, 1926) and the observations made in 1926 at different experimental stations, it will appear that fluctuations between different plants are considerable. For example, at Saratov in the extremely dry year 1924 the highest transpiration coefficient noted for flax was 1861 and the lowest transpiration coefficient for winter rye 202. In the more moist year 1925 the transpiration coefficients of different plants varied in a considerably smaller range from 859 for pumpkin to 123 for sorghum. In 1926 a smaller number of plants was studied from this point of view at Saratov, and the amplitude of fluctuations was as follows: the maximum transpiration coefficient noted for cicer was 884, and the minimum for winter rye 215. Two moist years, 1925 and 1926, have given at Saratov considerably closer values for the transpiration coefficients of similar plants than when either of these two years is compared with 1924. But the fluctuations in the figures of transpiration coefficients of similar plants are still present and are sometimes considerable.

If we take the extreme values of the transpiration coefficients for all plants in different years and divide the whole amplitude of fluctuations into three equal parts, all the plants will group themselves into three classes with respect to the magnitude of their transpiration coefficients. The first class will include plants with the highest, the second plants with the moderate, and the third plants with the lowest transpiration coefficients. Using this method the data for Saratov allow all plants to be classified into three such groups with respect to the magnitude of the transpiration coefficient during the period of three years.

Table VII.

Group	Transpiration coefficient	Year	Plants	Average
I	Maximum	1924	Flax, wheat Poltavka, pumpkin	1535
,,	,,	1925	Pumpkin, cicer	820
,,	,,	1926	Cicer, lence	839
II	Moderate	1924	Oats, barley, alfalfa, cicer	923
,,	,,	1925	Alfalfa, flax, barley, wheat Poltavka, millet, wheat Beloturka	448
,,	,,	1926	Sunflower, wheat Poltavka	536
III	Minimum	1925	Corn, sunflower, oats, potatoes, rye, lence, Sudan grass, peas, winter wheat, sorghum	240
,,	,,	1926	Sudan grass, corn, millet, rye	274

The plants of different experimental stations in 1926 were similarly classified by us, and then a table was made out for eight different plants which have during the period of these three years most frequently taken part in the experiments at different stations. This table shows the repeated presence of each of these plants in this or that group.

We repeat once more that the transpiration coefficients vary for different years and different stations, and that the classing of plants was only made by grouping according to the relative magnitudes of transpiration coefficients.

Table VIII.

Plant	Group I	II	III	Total number of cases
Spring wheat Poltavka	3	3	—	6
Corn	—	1	5	6
Winter rye	—	1	5	6
Sudan grass	—	2	4	6
Millet	—	3	3	6
Sunflower	1	1	3	5
Cicer	2	2	—	4
Lence	2	1	1	4

As shown by these figures spring wheat, beans and sunflower have most frequently the highest transpiration coefficient. On the contrary corn and winter rye have most often the lowest transpiration coefficient. Sunflower, millet and Sudan grass are often found in the last group with the lowest transpiration coefficient.

The data available on the transpiration coefficient of cultivated plants give thus the possibility of establishing that spring wheat is in this respect the most exigent plant. After it follow beans, millet and sunflower. On the other hand, corn, Sudan grass and winter rye must be classified as the less exigent plants. Corn and Sudan grass are followed by sorghum, winter rye, winter wheat, and potatoes.

3. *The use of water from the supply stored in the soil towards the time of sowing.* As shown by observations during the period of three years

different plants use during their growth different quantities of water from the initial water supply stored in the soil, supposing that all water supplied by rainfall during the period of growth is used by them. In the dry year 1924 all plants investigated at Saratov have used from the soil on the average 50 per cent. of total water needed. In the moist year 1925 the same plants have used on the average only 28 per cent. and in the likewise moist year 1926 this average was for all plants and at all experimental stations 38 per cent. On classing the plants into three groups according to the quantity of water used from the water supply stored in the soil, it will appear that early plants, flax and beans, will be usually found in the first group with the largest use of water from the soil. The late plants, pumpkin, potatoes, corn and millet, will be found in the last group with the lowest percentage of water used from the soil. The remaining plants investigated in that respect are found in the intermediate groups.

In the conditions of a dry region the early plants to have a successful growth must consequently find in the soil during the time of sowing considerably larger supplies of moisture. On the contrary, late plants are less interested in the initial water supply stored in the soil, as the largest portion of their requirement in water is supplied by rainfall during the period of growth.

CONCLUSION.

The problem of the use of water by cultivated plants in the field was first undertaken for study on the experimental field of the Field Husbandry Department at the Saratov Experimental Station. Two years later it was studied at six experimental stations of Nijnee Povoljie. The purpose of this collective experiment was to determine the character and the amount of the use of water by different plants under different weather conditions and on different soils.

The use of water was established by means of the determination, every ten days, of the water content in the soil beginning after sowing time and ending at or near harvest. The total quantity of precipitation during the period of growth was added to water lost from the soil during the period between the first and the last observation on soil moisture. The increase in the dry weight of the plant was determined at the same time. The total weight of water used from the soil (together with rainfall) was then divided by the dry weight of the crop, thus establishing the quantity called in this study "transpiration coefficient."

This investigation has enabled us to draw the conclusion that the

total quantity of water used in any year by different plants during their growth in different parts of the region and on different soils is more or less uniform. It depends to a considerable degree on the quantity of precipitation during the growth period and is, therefore, larger in moist years. When distinguishing in this total quantity the water used from the supply stored in the soil up to the time of sowing, and water supplied by rainfall during the period of growth, it should be noted that in dry years the larger portion of the plant's total requirement in water is obtained from the water supply stored in the soil, and *vice versa*. The comparison of different plants, in that respect, shows that to plants with a long period of growth the largest portion of their requirement in water is supplied by rainfall during the period of growth. The transpiration coefficients of different plants in the same year differ rather considerably from each other. In a dry year they are considerably higher than in a moist one. They are likewise considerably higher for early crops and beans and considerably lower for winter rye and wheat, corn, sorghum, Sudan grass, millet and potatoes. Or, stated in another way, they are lower for the plants with a long period of growth. From all plants observed at different stations during the period of three years the most exigent was spring wheat. It has most often the highest transpiration coefficient. The lowest transpiration coefficients have been shown more frequently by corn and winter rye.

The investigation confirms with sufficient precision the fundamental importance of the initial water supply stored in the soil for the plants with a short growth period as well as its considerably smaller importance for the late plants. It was not possible to determine any specific importance of the different types of soils at different experimental stations.

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A RAPID ELECTROMETRIC METHOD FOR MEASURING "LIME REQUIREMENTS" OF SOILS.

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I. Introduction.

The various methods that have been proposed for measuring the quantity factor in soil acidity, and for determining the lime requirements of acid soils, have been fully discussed in this *Journal* by Hutchinson and MacLennan (1), by E. A. Fisher (2), and, more recently, by Crowther and Martin (3). Whilst the need of a reliable and rapid laboratory method for determining lime requirements has long been felt, it is generally admitted that each of the procedures so far proposed is lacking in some particular, which renders it unsuitable either for routine soil examinations or for application to liming problems in the field. It is also generally admitted that the problem of elaborating an *entirely* satisfactory method for determining lime requirements in the laboratory is insoluble.

The addition of lime (calcium carbonate) to an acid soil produces the following main effects:

(*a*) *Effect of Ca-ion.*

1. Replaces exchangeable H-ion in the adsorption complex.
2. Replaces other cations (*e.g.* K-ion).
3. Reacts with PO_4-ion, SiO_3-ion and SO_4-ion of the soil-water system to form more or less insoluble compounds.

(*b*) *Effect of HCO_3-ion* (generated by carbonation).

1. Replaces exchangeable anions (*e.g.* PO_4-ion, SO_4-ion) in the adsorption complex.
2. Combines with H-ion (originally present in the aqueous phase, or liberated through replacement by Ca-ion in the colloidal complex) to form unionised H_2CO_3 molecules (a buffering effect).

(*c*) *Effect of OH-ion* (generated by hydrolysis).

1. Combines with H-ion to form unionised water molecules (neutralisation).
2. Replaces other anions in the adsorption complex.
3. Confers an alkaline reaction on the system when in excess.

It is evident that the correction of an acid soil condition by liming involves cationic and anionic exchange processes, as well as a true neutralisation process. Hence, for any lime-requirement method to be satisfactory, it must also involve and reproduce similar processes.

The Hutchinson-MacLennan method. One of the most commonly used methods for determining lime requirement is the Hutchinson-MacLennan method (1). Its applicability depends on the interaction between calcium bicarbonate in aqueous solution and the components of acid soils. Unfortunately, according to Crowther and Martin (3), the concentration of calcium bicarbonate recommended, namely 0·02 N, is such that the solution is initially acidic (pH 6·2), and may become much more acidic (*e.g.* pH 5·5) when finally in equilibrium with an acid soil. Furthermore, although the concentration of calcium-ion in the solution is relatively high, it is certainly not high enough to effect complete replacement of H-ion from the soil adsorption complex. Thus the Hutchinson-MacLennan method does not fully reproduce the various effects caused by liming a soil in the field.

Additional disadvantages of the method are that it is tedious and slow, and that it gives reproducible results only when the experimental conditions are strictly standardised. Thus, Crowther and Martin have shown that, by varying the amount of soil used and the initial concentration of the bicarbonate solution, values ranging from 0·548 to 0·179 per cent. of $CaCO_3$ could be obtained with a Harpenden Common soil.

In order to render the Hutchinson-MacLennan method more precise, Crowther and Martin recommend that three different quantities of soil, namely 10, 15 and 20 gm., be separately mixed with a certain volume of bicarbonate solution, and that the lime requirements derived from titration values of the filtrates be graphically interpolated to some arbitrary equilibrium bicarbonate concentration. They nevertheless found that satisfactory results could be obtained from a single determination by extrapolation to an arbitrary equilibrium concentration on a semi-logarithmic graph representing the relation between weight of soil and equilibrium concentration for any initial concentration of bicarbonate.

Electrometric methods for determining lime requirements. It has been amply demonstrated by E. A. Fisher (2), by Crowther and Martin (3), and by Christensen and Jensen (4) that titration (whether colorimetric or electrometric) of soil suspensions with alkaline solutions, such as soda, baryta or lime-water, does not furnish a suitable method for

determining lime requirements, since it mainly assesses, by neutralisation, the quantity factor of soil acidity, and only very partially the capacity of a soil for exchange with calcium-ion.

By adding a neutral salt (calcium chloride) to calcium hydroxide or bicarbonate solution used for electrometric titration, Crowther and Martin found, however, that much higher lime-requirement values were obtained than when the alkaline solution alone was used. This result is to be expected, since the neutral salt addition considerably increases the calcium-ion concentration of the solution, so that its capacity for cationic exchange becomes greatly enhanced. In particular, the addition of neutral salt notably increases the capacity of the reagent for displacing hydrogen-ion from the soil adsorption complex, and thus rendering it amenable to direct titration.

These observations and results suggest that the regular inclusion of a neutral salt in electrometric titrations of acid soils with alkalies might furnish a reliable lime-requirement method. The following account describes a procedure which has given every satisfaction, and which, though admittedly not perfect, possesses the great advantages of simplicity and rapidity in use.

II. EXPERIMENTAL.

The method finally evolved consists first in the development of "exchange acidity," by treating a known mass of the soil under examination with a solution of calcium chloride, and then titrating the mixture directly with dilute standard lime-water until its *p*H value reaches 7·0, as determined by the quinhydrone electrode.

Note. The reaction mechanism of this procedure is similar to that of the method of Daikuhara (5), in which potassium chloride is used as neutral salt. In the Daikuhara method, as in other early methods in which neutral salt treatment is applied, the treated soil is decanted or filtered, and the titration with alkali performed on the clear liquid. In the writers' method, titration is performed *directly* on the salt-soil mixture, an operation which the employment of the quinhydrone electrode readily allows.

Trénel (6) has recently described a method for determining lime requirements, in which soil, suspended in 0·1 molar potassium chloride solution, is titrated electrometrically with standard caustic soda to any desired *p*H value measured by the quinhydrone electrode, but it is not quite clear from his description whether or not the titration is performed directly on the salt-soil mixture, or on its filtrate. As far as the writers

are aware, no detailed method so far has been proposed in which the titration is carried out in the manner described in the following account.

Choice of neutral salt. In order to avoid complications in the cationic exchange process, calcium chloride was selected as neutral salt in preference to potassium chloride, sodium chloride or barium chloride. The pure salt was dissolved in distilled water in amount sufficient to give a solution of one-fifth molar (0·2 *M*) concentration. More concentrated salt solutions cannot be employed in electrometric titrations involving the use of quinhydrone, otherwise serious "salt error" may be introduced (7). The calcium chloride solution was previously accurately neutralised to *p*H 7·0 by the aid of brom-thymol-blue, checked by the quinhydrone electrode. A sufficient quantity of 0·2 *M* $CaCl_2$ solution was made up to serve the needs of a large number of lime-requirement determinations.

Proportion of soil to salt solution. In all the experiments, 10 gm. of air-dry soil, previously passed through a 1 mm. sieve, were mixed with 40 c.c. of neutral 0·2 *M* $CaCl_2$ solution by shaking in a 150 c.c. solid flat-stoppered, wide-mouthed, hard glass bottle. The mixture was usually allowed to stand overnight in order to develop the exchange acidity. Theoretical considerations and actual trial proved this to be unnecessary, since no appreciable differences in *p*H value of the mixture could be detected in the majority of cases after periods of contact ranging from one minute to several days. In the case of soils containing traces of free lime, however, a rather lengthy period of contact is probably advisable.

Titration procedure. A sufficient quantity of quinhydrone was added to the soil-salt solution mixture, and the *p*H value determined by the quinhydrone electrode. Successive portions of lime-water of known concentration (0·03 *N* was used) were then added from a burette supplied with the solution from a large reservoir furnished with a protecting soda-lime tube. Between each addition the contents of the bottle were shaken by hand vigorously and continuously for a time.

The reaction was determined after shaking by means of the quinhydrone electrode. It was recorded as millivoltage measured against a saturated calomel half-cell. When the *p*H value of the mixture had overstepped neutrality (*p*H 7·0), the titration was considered complete. Lime requirements were deduced by plotting the millivolt readings of the potentiometer against volumes of standard lime-water required to give a final reaction of, say, *p*H 7·0. (Actually in the writers' experiments a final reaction of *p*H 7·2 was favoured.)

A series of trials were run in which the volume of the successive additions of lime-water, and also the time of shaking between each addition, were varied. Some typical results are recorded in Table I. The data were obtained in a series of experiments in which the volume of each addition of lime-water was 3 c.c., but in which four different reaction times were allowed before the pH value was determined.

Table I. *Effect of time of contact between salt-treated soil and added lime-water.*

(10 gm. of soil, 40 c.c. of 0·2 M $CaCl_2$, titrated with 0·03 N $Ca(OH)_2$.)

Vol. of $Ca(OH)_2$ added (c.c.)	Period of contact: Shaken 1 min.		Shaken 3 min.		Shaken 10 min.		Standing 24 hr.
Soil (A), Caroni. (Humus, nil.)							
0	pH 4·43	4·43	pH 4·43	4·43	pH 4·43	4·43	pH 4·43
3	5·17	5·13	5·00	5·00	4·95	4·95	4·80
6	5·77	5·73	5·51	5·52	5·42	5·42	5·18
9	6·20	6·18	5·93	5·95	5·83	5·83	5·67
12	6·52	6·50	6·25	6·27	6·12	6·12	5·88
15	6·80	6·78	6·53	6·54	6·38	6·38	6·13
18	7·03	7·03	6·78	6·79	6·63	6·63	6·33
21	7·23	7·22	6·97	6·98	—	—	—
24	7·40	7·38	7·17	7·18	—	—	—
27	—	—	7·33	7·34	—	—	—
Soil (B), Felicity. (Humus, 2·4 %.)							
0	pH 5·45	5·45	pH 5·45	5·45	pH 5·45	—	pH 5·45
3	5·98	5·99	5·78	5·78	5·73	—	5·72
6	6·38	6·38	6·08	6·08	5·96	—	5·93
9	6·68	6·69	6·33	6·33	6·16	—	6·03
12	6·90	6·91	6·55	6·56	6·35	—	6·18
15	7·08	7·08	6·73	6·73	6·48	—	6·30
18	7·25	7·23	6·90	6·90	6·78	—	6·47
21	7·37	7·33	7·05	7·06	—	—	6·50
24	—	—	7·18	7·19	—	—	6·67
27	—	—	7·30	7·31	—	—	6·77

The data presented in Table I demonstrate (a) that reproducible results in the titration of any given salt-treated soil can readily be obtained, and (b) that only a slight change in reaction (which would be due to further displacement of H-ion by Ca-ion of the added lime-water) occurs after a three minutes' shaking period. There is little advantage to be gained, therefore, in lengthening the time of continuous shaking beyond this interval.

Data showing the effect of varying the volume of each addition of lime-water, keeping constant the intermediate shaking periods, are not presented. Definite trials of this sort indicated that (a) there was no advantage in lessening the volume of each addition below 5 c.c., but that (b) additions of volume greater than 5 c.c. necessitated increases in the duration of the time of shaking.

Table II. *Comparison of electrometric method with Hutchinson-MacLennan method for soils of different textures and reaction values.*

1	2	3	4	5	6
			Lime requirement % $CaCO_3$		
Soil No.	Index of texture*	Exchange reaction† (pH)	(A) Electro-metric method	(B) Hutchinson-MacLennan method	Ratio $\frac{A}{B}$
I. Sands.	(0–25)				
WO XXV	19	3·85	0·226	0·131	1·73
CA XXI	20	4·00	0·307	0·413	0·74
CA I	21	4·38	0·178	0·223	0·80
WO XXXI	18	4·47	0·215	0·144	1·88
WO I	23	4·52	0·186	0·105	1·77
O I	25	4·75	0·146	0·171	0·85
WO II	16	5·27	0·120	0·023	5·22
CA II	20	5·55	0·111	0·166	1·50
CA XVIII	23	6·03	0·097	0·023	4·22
CA III	23	6·53	0·046	0·068	0·67
O IV	24	7·35	0·000	0·041	—
II. Loams.	(25–40)				
CA XXII	32	3·88	0·455	0·580	0·78
M VI	37	4·30	0·374	0·251	1·49
CA IV	35	4·38	0·298	0·317	0·94
CA XII	33	4·53	0·242	0·276	0·87
WO III	33	4·83	0·256	0·153	1·67
CA XIII	29	5·25	0·153	0·185	0·82
O VI	36	5·32	0·141	0·068	2·07
CA V	32	5·58	0·124	0·172	0·72
O VII	36	6·20	0·093	0·049	1·89
M VII	37	6·37	0·114	0·049	2·32
O VIII	38	7·12	0·034	0·000	—
III. Silts.	(40–50)				
CA XV	45	3·80	0·601	0·569	1·06
CA VI	49	3·92	0·632	0·543	1·16
CA XVI	40	4·15	0·357	0·358	1·00
M VIII	43	4·50	0·389	0·367	1·06
CA VII	44	4·50	0·334	0·299	1·12
O IX	42	4·57	0·259	0·218	1·19
WO IV	47	4·97	0·343	0·191	1·79
WO V	44	5·52	0·175	0·666	2·65
O X	40	5·63	0·152	0·102	1·49
CA VIII	41	5·92	0·126	0·125	1·01
IV. Clays.	(50–60)				
CA XX	51	3·83	0·776	0·722	1·08
CA IX	53	3·87	0·711	0·616	1·15
CA X	51	4·25	0·447	0·393	1·13
WO V A	54	5·17	0·339	0·194	1·74

* Moisture at point of stickiness *minus* 0·2 × (coarse and fine sand). See Hardy, F., *J. Agric. Sci.* **18** (1928), p. 252.

† Determined by quinhydrone electrode on 1 : 4 suspension in molar KCl solution.

By adopting as standard procedure a three minutes' period of shaking, together with 5 c.c. successive additions of lime-water of

0·03 normality, it was found possible, with the aid of an assistant, to run titrations on three different soils at once. The average time required for a single determination was about 10 minutes when ten to twenty soils were set up for examination on any one day. Thus the method proved to be very suitable for routine laboratory work.

III. Comparison with the Hutchinson-MacLennan Method.

In Table II are presented the results obtained in lime-requirement determinations by the electrometric method described above and by the Hutchinson-MacLennan method, applied exactly as directed by its originators, but with none of the refinements suggested by Crowther and Martin.

The soils are set out in the table in four textural groups, and within each group they are arranged in the order of their exchange reactions (pH values of 1 : 4 suspensions of approximately molar potassium chloride solution).

The data in Table II suggest the following generalisations:

(1) The electrometric method yields much more regular and orderly results than the Hutchinson-MacLennan method. Thus, it shows quite clearly the proportionate increase in lime requirements (*a*) of soils of approximately the same initial exchange reaction but of increasing degree of fineness of texture, and (*b*) of soils of approximately the same texture but of increasing exchange pH value. Slight irregularities within any one textural group may be ascribed to variations in texture within that group.

(2) The electrometric method yields results that are generally numerically greater than those given by the Hutchinson-MacLennan method. This is in accordance with expectation.

In 26 cases out of 36 it gave larger results, and in five of these the results were more than twice as large. The discrepancies are believed to be mainly due to the greater experimental error in the Hutchinson-MacLennan method. That they were not due to differences in content of humus was readily proved by comparison with actual analytical data not recorded in the table. The closest agreement between the two methods was given by the finer types of soils, namely the silts and clays.

IV. Comparison with the Base Exchange Method.

Several procedures for determining lime requirements of soils as saturation deficits in the quantity of exchangeable calcium present have recently been described. From certain points of view the base

exchange method is fundamentally the most sound of all. Nevertheless, in practice, the final reaction attained at 100 per cent. saturation is not usually that corresponding to neutrality, so that the method fails to assess accurately the intensity factor in soil acidity. Thus Hissink (8) has shown that, at *p*H 7·0, many Dutch soils are only 55 per cent. saturated, and Bradfield (9) has found that 100 per cent. saturation may not be attained until a reaction between *p*H 10 and *p*H 11 is reached.

Through the courtesy of Mr P. E. Turner, the writers have been able to compare for a large number of soils the values obtained by the electrometric lime-requirement method with those obtained by a base exchange method devised by him. Presentation of the data and discussion of the results are reserved for an article now being prepared by Mr Turner. In general, the base exchange method yielded values that were in considerable excess of the electrometric figures. The average ratio between them approximated to 2·0, and the range of difference to between 0·7 and 9·4 per cent. of lime (CaO).

It is evident that much further work is needed before an ideal lime-requirement method can be formulated for laboratory use. Even if attained, such an ideal laboratory method may require serious modification if it is to satisfy the exacting demands of field practice.

Summary.

1. A method for the rapid determination of lime requirements in laboratories equipped with the quinhydrone electrode is described. (10 gm. of soil are mixed in a small wide-mouthed bottle with 40 c.c. of neutral 0·2 *M* $CaCl_2$ solution. The mixture is then titrated with 0·03 *N* lime-water in successive portions of 5 c.c., with three minutes' shaking between each addition. The *p*H value of the mixture is determined after each addition, and the titration continued until the reaction has passed *p*H 7·0. The results are plotted, and the exact volume of lime-water needed to give a final reaction of *p*H 7·0 is estimated from the graph.)

2. The method is compared with the Hutchinson-MacLennan method for a series of soils of different textures and different initial exchange reactions. It appears to yield more reliable results; it is less tedious; it is very rapid, and results obtained thereby can readily be reproduced.

3. A comparison of results obtained by the method with those obtained by a base exchange (lime status) method is shortly stated.

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THE LIME STATUS OF SOIL IN RELATION TO AN INSECT PEST OF SUGAR-CANE.

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I. Introduction.

For some years sugar-cane (*Saccharum officinarum* L.) in Trinidad has been subject at irregular intervals to serious damage by a complex of diseases known locally as "froghopper blight." The nature of the injury sustained by the plant has been described by Williams (8) as "a browning and drying-up of the leaves of the cane, and a decay of the root system, which results in a more or less complete cessation of growth, an appearance throughout the field as if the canes had been scorched by fire, and a loss ranging from a slight check in growth to a complete destruction of the crop." The blight is primarily caused by the sucking action on the cane leaves of a Cercopid, *Monecphora* (*Tomaspis*) *saccharina* Dist., commonly known as the "froghopper," but the extent of the injury may be increased materially by the attacks of root fungi (*Marasmius* and *Odontia*). Various aspects of the disease have been studied by Williams (8) and by Withycombe (9), to whose works reference should be made for further details.

The prevalence and intensity of blighting are influenced by many secondary factors, the chief of which are the amount and distribution of the rainfall, the condition of the soil, and the age and variety of the cane. Infestation by froghoppers is usually mild when the rainfall is evenly distributed, but it tends to be severe when marked alternations of wet and dry periods occur. Blighting is then most serious on "ratoon[1]" canes growing on badly drained areas and on soils in poor condition.

Various attempts have been made in Trinidad to associate individual soil factors with the susceptibility of the cane to damage from froghoppers. Carmody (1) and Urich (7) have shown that the soils of certain blighted regions possess a high ratio of total magnesium to total calcium. Williams (8) discovered that many of the unblighted areas are alkaline or neutral to litmus, whilst those of the blighted regions are frequently

[1] The sugar-cane is propagated from sections of the stem, which give rise to the original "plants." When mature they are cut flush with the ground, sprouting again to give "first ratoons." Ratooning may be continued for some years.

acidic, but he found too many exceptions to warrant a generalisation. He pointed out that the alkaline and neutral soils contain, on the whole, larger amounts of calcium, and are more open and looser in texture than the acid soils.

Hardy (3), using the quinhydrone electrode, has further examined the connection between soil acidity and the susceptibility of the sugar-cane to blight. In a thorough survey of several of the cane-growing estates of the island, he has demonstrated that, in general, soils invariably supporting unblighted canes are not only alkaline in reaction, but usually possess exchange alkalinity, *i.e.* remain alkaline in the presence of a strong neutral salt solution. He found that blighted canes are usually supported by acid soils, the more acid the soil, the greater the susceptibility of the cane to damage. Re-examination by Hardy of the soils believed by Williams to be exceptions gave additional confirmation to the soil reaction theory.

II. The Soils Examined.

The soils of the cane-growing regions fall naturally into two distinct areas, the Naparima District and the Central Alluvial Plain. These areas differ greatly in character, and are therefore discussed separately.

The Naparima District contains a number of distinct soil types, which may be classified as follows:

Group I. *Blighted Types.*

A. A brown soil type, characterised by its subsoil, a bluish-green clay, which frequently contains calcium carbonate.

B. A red soil type, derived from a slate-blue clay devoid of calcium carbonate.

The soils of this group have been continuously under sugar-cane cultivation for a very long period. They are blighted whenever an infestation of froghoppers occurs, but the canes on the red soil suffer more severely than those on the brown soil.

Group II. *Blight-free Types.*

D. A dark brown alluvial soil type, on which sugar-canes have been grown in recent years only.

E. A black soil type, which overlies calcareous marl. It has supported sugar-canes for generations, but still contains a reserve of calcium carbonate.

Group III. *Blighted and Blight-free Types.*

C. A yellow-brown alluvial soil type, which may be divided into two

sub-types, one of which is habitually blighted, and the other invariably blight free. It is classified in a group of its own, as it alone, of the various soil types present in the Naparima District, affords sites, within a single type, suitable for the investigation of the variation in soil factors possibly associated with the susceptibility of the cane to froghopper injury.

The Naparima District is undulatory in topography, but the incidence of blighting is independent of the contour of the land (8). Where blighted and unblighted contiguous areas occur, they are usually separated by a well-defined line of demarcation which coincides exactly with the junction of the soil types of the first and second groups.

The soils of these contrasted groups differ strikingly in physical condition. Those of Group I, and the blighted sub-type of Group III, are characterised by a badly impaired tilth, due in part to exhaustive cropping. In dry weather they set like concrete, and when the rains begin they assume the consistency of putty. They appear to be poorly aerated, for the sub-surface soil visibly changes colour on exposure to air. The soils of Group II, and the blight-free sub-type of Group III, show much less variation throughout the year. In the dry season they are more open and friable, and in wet weather they retain a fair physical condition.

The Central Alluvial Plain is flat in character and contains no well-defined soil types. The soils vary irregularly in texture from light sands to heavy clays. The whole plain, with the exception of a few small areas, has been blighted at one time or another. The heavier blighted and blight-free soils differ in their properties in a manner similar to the soils of the Naparima District. A tendency to cement in dry weather and a high degree of acidity, or extreme lightness in texture, are the predominating characteristics of the lighter soils which support canes susceptible to froghopper injury.

III. Some Constants of the Soils Examined.

The reaction of the sugar-cane to froghopper attack appears to be so closely related to the field behaviour of the soil that a comparison has been made of some of the more important physical and physico-chemical constants of typical blight-free and blighted soils. The investigations were performed on composite samples of twelve borings representative of small areas within unblighted or uniformly blighted regions. To avoid, as far as possible, the complications introduced by contour and water-logging, the samples were taken on the flat, at points where the drainage appeared to be good. In the Naparima District, which was first examined,

Table I. *Naparima District. Constants of blighted and blight-free soils.*

	Sample depth (feet)	Blighted soils, Group I, A: M 50	A: MH 15	B: M 179	B: MPD 9	Group III, C: M 38	C: M 151	Blight-free soils, Group III, C: M 64	C: M 100	Group II, D: M 40	D: M 190	E: M 42	E: MPD 7	Blighted soils Mean value	Blight-free soils Mean value
1. Clay + fine silt II	0–1	62·67	74·60	67·62	57·50	37·80	51·59	45·32	51·00	63·60	67·50	60·00	53·05	58·63	56·75
	1–2	68·25	76·20	75·65	63·02	42·25	57·75	48·70	58·53	65·00	71·05	62·80	58·52	63·85	60·77
2. Index of texture (4)	0–1	41·1	39·4	42·1	41·0	27·5	40·0	33·1	36·3	46·3	45·1	47·1	43·9	38·5	42·0
	1–2	34·3	42·9	38·0	37·6	32·1	39·1	33·2	38·4	40·4	40·6	55·8	37·8	37·3	41·0
3. Organic matter	0–1	4·6	3·5	2·5	4·3	2·7	3·8	4·2	5·0	5·2	5·1	6·8	6·0	3·6	5·4
	1–2	2·2	3·3	2·3	5·6	2·7	3·0	3·7	2·8	4·8	3·6	5·0	5·2	3·2	4·2
4. $CaCO_3$	0–1	0·00	0·00	0·00	0·00	0·00	0·00	0·28	0·15	0·00	0·00	Trace	0·14	0·00	0·95
	1–2	0·00	0·00	0·00	0·00	0·00	0·00	0·00	Trace	Trace	0·00	Trace	Trace	0·00	Trace
5. *p*H	0–1	5·04	5·31	4·72	5·03	5·32	5·20	7·14	6·61	5·69	6·39	5·84	7·48	—	—
	1–2	5·51	5·34	4·38	4·56	5·12	4·79	5·58	7·51	6·31	6·98	6·51	6·94	—	—
6. Exchangeable calcium (per 100 gm. air-dry soil)															
(*a*) gm. CaO	0–1	0·431	0·401	0·210	0·315	0·245	0·307	0·470	0·463	0·810	0·622	1·046	0·919	0·318	0·722
	1–2	0·538	0·389	0·197	0·241	0·235	0·228	0·349	0·547	0·870	0·652	1·090	0·866	0·305	0·729
(*b*) mg. E.	0–1	15·39	14·32	7·50	11·25	8·75	10·96	16·80	16·54	28·93	22·21	38·07	32·82	11·36	25·90
	1–2	19·21	13·89	7·04	8·61	8·39	8·14	12·46	19·54	31·07	23·29	38·93	31·64	10·89	26·16
7. Ratio. Ex. Ca/(C. + F.S. II)	0–1	0·25	0·19	0·11	0·20	0·23	0·21	0·37	0·32	0·45	0·33	0·63	0·62	0·20	0·45
	1–2	0·28	0·18	0·09	0·14	0·20	0·14	0·26	0·33	0·48	0·33	0·62	0·54	0·17	0·43

Table II. *Central Alluvial Plain. Constants of blighted and blight-free soils.*

	Blighted soils: WOA 341	WOPD 5	CPD 3	W 85	WOPD 3	W 106	CA 2	CA 1	Blight-free soils: A 10	O 122	CPD 1	CA 16	O 59	CA 15	W 48	Blighted soils. Mean value	Blight-free soils. Mean value
1. Clay + fine silt II	7·12	55·12	52·02	49·75	51·62	70·00	75·75	77·90	18·57	29·10	22·03	23·99	36·25	55·17	56·00	61·72	34·44
2. Index of texture (4)	5·0	34·6	32·6	30·8	31·6	42·7	42·8	46·3	20·1	31·2	16·7	17·2	28·2	32·9	34·2	37·3*	25·8
3. Organic matter	1·8	4·8	2·8	2·8	2·9	4·0	5·5	6·0	2·5	3·0	2·7	2·1	3·2	6·8	2·8	3·8	3·3
4. $CaCO_3$	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	Trace	0·00	Trace	Trace	Trace	Trace	0·00	0·00	Trace
5. *p*H	6·06	4·91	6·30	4·85	5·06	4·72	4·77	4·70	7·66	6·89	7·87	7·44	6·91	6·83	6·68	—	—
6. Exchangeable calcium (per 100 gm. air-dry soil)																	
(*a*) gm. CaO	0·094	0·222	0·309	0·284	0·179	0·315	0·380	0·364	0·205	0·230	0·256	0·279	0·369	0·439	0·472	0·268	0·321
(*b*) mg. E.	3·36	7·93	11·04	10·14	6·39	11·25	13·57	13·00	7·32	8·21	9·14	9·96	13·18	15·68	16·86	9·58	12·91
7. Ratio. Ex. Ca/(C. + F.S. II)	0·47	0·14	0·21	0·20	0·12	0·16	0·18	0·16	0·39	0·28	0·41	0·42	0·36	0·28	0·30	0·17*	0·35

* Excluding WOA 341.

the top and second foot of soil were investigated. On the Central Alluvial Plain samples were obtained of the top foot only. The data secured are summarised in Tables I and II.

The results of mechanical analyses of the soils of the Central Alluvial Plain indicate that the blight-free areas include a smaller proportion of extreme mechanical types than the blighted areas. In the Naparima District, however, the soils of the contrasted areas rarely exhibit significant differences in their mechanical composition. Almost all the soils of this region contain sufficient clay and very fine silt to place them in an extremely heavy class. The data obtained show that, although the mechanical composition of the soils may be a contributory cause both of the dissimilarity in their own properties and those of the canes they support, it cannot be regarded, except for soils of the lightest texture, as a factor of primary importance. The indices of texture (4) of the soils confirm this conclusion.

No striking differences appear in the soil contents of organic matter[1]. The amounts present in the unblighted soils of the Central Alluvial Plain are frequently smaller than those in the blighted soils. The blight-free soils of the Naparima District contain, on the whole, slightly larger quantities than the blighted soils, but the differences are, as a rule, inadequate to account for the manifestly inferior attributes of the latter.

The blighted and blight-free soils of both areas differ, however, in that whereas the former are devoid of calcium carbonate, the latter almost invariably contain at least traces of this substance. The quantities of carbonate found in the unblighted soils are usually appreciably larger than those recorded in Tables I and II. In the selection of samples for examination, those with the smallest contents were chosen, to reduce to a minimum the error involved in the determination of the soil content of exchangeable calcium.

The *p*H data, determined by the use of the quinhydrone electrode, confirm the results of Hardy (3). The soils of the blighted areas of the island are invariably acid, the majority possessing a high content of exchangeable hydrogen ions. Those of the blight-free areas are markedly more alkaline, and though frequently somewhat acidic in character, they rarely exhibit extreme acidity.

The major differences in the measured soil constants appear, therefore, to be confined to the reaction of the soil and the presence or absence of calcium carbonate. Such differences are known to be correlated with the soil content of exchangeable calcium, which has been determined.

[1] The soil contents of organic matter were determined by the peroxide method (6).

Various investigators, notably Gedroiz (2) and Hissink (5), have shown that the properties of normal soils depend largely on the amount of exchangeable calcium they contain.

IV. THE CONTENTS OF EXCHANGEABLE CALCIUM OF THE BLIGHTED AND BLIGHT-FREE SOILS.

The Naparima District. The contents of exchangeable calcium of the contrasted soils of the Naparima District are such as to indicate that a close relationship exists between these amounts, the field behaviour of the soil, and the susceptibility of the cane to froghopper injury. The soil types D and E of Group II, which are in good condition and blight free, have a mean content of 25·57 and 35·45 mg. E. per cent., respectively, for the top foot, and 27·18 and 35·29 mg. E. per cent., for the second foot. The corresponding values for the equally heavy soil types A and B of Group I, which possess an impaired tilth and are subject to blight, are 14·86 and 9·38 mg. E. per cent. for the topsoils and 16·55 and 7·83 mg. E. per cent. for the subsoils. Of the two soil types of this group, type B, which supports canes habitually more severely blighted than type A, has the lower content of exchangeable calcium. The relationship holds equally well for the contrasted sub-types of soil type C. The lowest values for the top and second foot of the unblighted sub-type are 16·54 and 12·46 mg. E. per cent. The highest values for the blighted sub-type are 10·96 and 8·39 mg. E. per cent.

The relationship still holds when allowance is made for the variation in the mechanical composition of the soils. The values of the ratio of exchangeable calcium to clay and fine silt for the topsoils and subsoils of the blight-free regions lie between 0·32 and 0·63 and 0·26 and 0·62 respectively. The corresponding values for the soils of the blighted regions range from 0·11 to 0·25 and 0·09 to 0·28. Although the subsoil content of exchangeable calcium may not be as important as that of the topsoil, the amounts present in the former, and its ratio values, almost always compare favourably with those of the latter, in the unblighted areas of the Naparima District.

The Central Alluvial Plain. The mean content of exchangeable calcium of the unblighted soils of the Central Alluvial Plain is slightly larger than that of those supporting canes susceptible to blight. No relationship, however, appears to exist between the quantities present in individual samples, the physical condition of the soils of which they are representative, and the resistance of the cane to disease. The soils which are readily maintained in a favourable state, and support canes resistant

to blight, contain from 7 to 17 mg. E. per 100 gm. of air-dry soil. Those in poor condition, which grow readily blighted canes, possess from 3 to 14 mg. E. per cent. Apparently the tilth of the soil may or may not be impaired, and froghopper damage may vary greatly, between the limiting values of 7 and 14 mg. E. per cent.

This lack of relationship disappears when account is taken of the varying amounts of clay and very fine silt present in the soils examined. The maximum value of the ratio for the soils of the blighted regions (excluding sample WOA 341) is 0·21, whilst the minimum value for the blight-free soils is 0·28. These values differ but little from the largest and smallest ratios respectively for the blighted and unblighted regions of the Naparima District. The soil WOA 341 is peculiar because of the extreme lightness of its texture. It is insufficiently colloidal to support cane growth successfully under the prevailing climatic conditions.

V. The Relation between the Soil Content of Exchangeable Calcium and the Intensity of Blighting.

The ratio of the content of exchangeable calcium to clay and very fine silt for the blight-free soils differs sufficiently markedly from that for the blighted soils to differentiate them clearly. In view of the very great variation which occurs in the conditions under which the sugar-cane exists in the sites from which the samples were taken, the data collected not only indicate that the lime status of the soil is of primary importance, but suggest that, under circumstances in which the other factors associated with the reaction of the cane to froghopper attack are constant, a positive correlation may exist between the relative amount of exchangeable calcium in the soil and the intensity of injury sustained by the cane. To investigate this possibility, an examination was made of two small areas of the Central Alluvial Plain, in each of which the general field conditions, the texture of the soil and the variety and age of the cane were uniform, but on which the canes exhibited varying degrees of froghopper damage. The results obtained are recorded in Table III. The estimate of the injury to the canes on one area was confirmed by the determination of the yields. The yields for the other area are not available.

The data secured certainly indicate that for these areas, a diminution in the soil content of exchangeable calcium is accompanied by increased intensity of injury. It is worthy of mention that the physical condition of the soils on which the canes were very severely blighted appeared to be much less favourable than the condition of those supporting lightly blighted canes.

Table III. *Relation between intensity of blighting and soil content of exchangeable Calcium.*

Index no. of soil	pH	Exchangeable calcium per 100 gm. of air-dry soil: Gm. CaO	Mg. E.	Intensity of blighting	Yield of cane (tons per acre)	Variety and age of cane
Area I. (C. + F.S. II = 38·2)						
WOA 320	6·41	0·301	10·75	Slight	$25\frac{1}{4}$	B 156 first ratoons
WOA 339	6·14	0·251	8·96	Slight	$25\frac{3}{4}$	B 156 first ratoons
WOA 332	6·49	0·194	6·93	Moderate	21	B 156 first ratoons
WOA 295	5·86	0·104	3·71	Very bad. Canes almost destroyed	3	B 156 first ratoons
WOA 272	4·79	0·085	3·04	Very bad. Canes destroyed	1	B 156 first ratoons
Area II. (C. + F.S. II = 54·0)						
W 48	6·68	0·472	16·86	Slight	—	BH 10/12 first ratoons
W 62	6·06	0·455	16·25	Fair	—	BH 10/12 first ratoons
W 85	4·84	0·284	10·14	Moderate	—	BH 10/12 first ratoons

VI. Discussion.

There seems little doubt that the soils of the regions liable to frog-hopper blight are lacking in exchangeable calcium. The effect of such a deficiency may be direct, in that it leads to calcium starvation of the plant, or secondary, by reason of its influence on the physical condition of the soil.

The indirect effect probably predominates on soils possessing more than 7 mg. E. per cent. of exchangeable calcium, the minimum content of the blight-free soils. The fact that many blighted soils contain much larger quantities, but that a relationship exists between the soil content of exchangeable calcium and the reaction of the cane to froghopper attack when allowance is made for the mechanical composition of the soil, suggests that on them the tilth factor is of primary importance.

Experience gained by liming affords evidence in support of this view. Applications of ground limestone to soils containing more than 7 mg. E. per cent. of exchangeable calcium, in amounts sufficient appreciably to increase this value, but inadequate to cause amendment of the physical condition of the soil, have not enhanced the resistance of the sugar-cane to froghopper injury. On the other hand, soils at one time in poor condition and blighted, which have been limed until they are in good tilth, are now blight free.

When the amount of exchangeable calcium present in the soil falls below 7 mg. E. per cent., there are indications that calcium starvation of the sugar-cane may occur. The canes growing on the sites from which the samples WOA 272 and WOA 295 were obtained are habitually yellow and stunted, and make poor growth compared with those of

neighbouring areas less liable to blight. These samples contain less than 4 mg. E. per cent. of exchangeable calcium. The canes on such soils are so severely blighted when infestations of froghoppers occur that they are almost completely destroyed. Further, the data in Table III show that the damage sustained by the cane increases markedly when the percentage of exchangeable calcium falls below 6·9 mg. E.

SUMMARY.

1. The sugar-cane in Trinidad is subject to blight, caused primarily by the sucking action on its leaves of a Cercopid, *Monecphora* (*Tomaspis*) *saccharina* Dist., commonly known as the "froghopper." The intensity of the injury sustained by the cane appears to depend largely on the condition of the soil in which it is grown. An investigation has, therefore, been made of the extent to which certain soil factors are associated with the reaction of the cane to froghopper attack.

2. The data obtained show that the mechanical composition of the soil and its content of organic matter bear little relationship to the damage caused. The blighted soils differ from those blight-free, however, in that, whereas the former are devoid of calcium carbonate and, as a rule, markedly acid, the latter almost invariably contain at least traces of this substance, and in general are alkaline or slightly acid only. These differences suggest that the lime status of the soil is a factor of primary importance.

3. Determination of the contents of exchangeable calcium of typically blighted and blight-free soils has confirmed this view. In spite of the great variations in the conditions under which the sugar-cane exists in the sites from which samples were taken, the contrasted soils may be distinguished by the value of their ratios of exchangeable calcium to clay and very fine silt. The ratio value for the topsoils of the unblighted areas varies from 0·28 to 0·63, that for the subsoils from 0·26 to 0·62. The corresponding ratios for the topsoils and subsoils of the habitually blighted areas range from 0·11 to 0·25 and 0·09 to 0·28, respectively. The contents of exchangeable calcium, and the ratio values of the subsoils of the unblighted areas examined, almost always compare favourably with those of the topsoils.

4. In areas of uniform soil texture in which the other factors associated with the susceptibility of the sugar-cane to injury are approximately constant, a very close relationship exists between the soil content of exchangeable calcium and the damage sustained by the cane.

5. There is evidence that in soils containing more than 7 mg. E. per

cent., the effect of the soil deficiency in exchangeable calcium on the reaction of the cane to froghopper attack is mainly indirect, by reason of its influence on the physical condition of the soil. Where the soil content falls appreciably below this value, the data obtained indicate that calcium starvation of the cane may contribute to the damage it sustains.

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STUDIES IN MILK SECRETION BASED ON THE VARIATIONS AND YIELDS OF MILK AND BUTTER FAT PRODUCED AT MORNING AND EVENING MILKINGS.

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(With Five Charts.)

Material.

Since January 1922 the milk of each cow in the dairy herd belonging to the National Institute for Research in Dairying has been tested for butter fat at each milking on three consecutive days (usually Tuesday, Wednesday and Thursday) about the middle of each calendar month. It was thought that the material so collected, if analysed, would provide information of some value for studying various aspects of problems relating to the fat in cows' milk and might assist in pointing to suitable lines along which further experiments could be planned to increase our knowledge of milk secretion.

During the five years' period 1922 to 1926 over 10,000 samples of milk have been tested for fat and 111 complete lactation records of cows have become available for analysis. Of these lactation records 97 were obtained from non-pedigree Dairy Shorthorns and 14 from pedigree Guernsey cows. The herd was managed under conditions common in the south of England, *i.e.* the cows grazed pasture night and day during the summer (May to October), the grass being supplemented by green fodder from arable land when necessary, and concentrates were fed to the heavier milkers according to their yield. During the winter (November to April) the cows were housed at night and, except during bad weather, ran on pasture land by day, practically all foods were fed indoors and therefore easily controlled in quantity and quality. The feeding standards employed for rationing were those recommended by Mackintosh which maintained the cows in good thriving condition. The health of the herd was excellent throughout the period, the only illnesses experienced being occasional lameness and a few isolated instances of mastitis.

Sampling for the three-day monthly tests was always carried out by a responsible person, the milk of each cow being mixed by pouring from pail to pail three times, immediately after milking; the samples were

tested in the chemical department of this Institute, the Gerber method being employed using calibrated glassware and testing the milk in a fresh condition without preservative; the accuracy of the results were verified from time to time against gravimetric methods.

The intervals between milkings have not varied to any appreciable extent during the five years, the night interval being 15¼ hours and the day interval 8¾ hours. During the later years it has been the usual practice to milk the cows giving the largest quantities of milk first in the morning and last at night, thus, while cows are at their maximum yield the intervals tend to be slightly more equal than those times mentioned and when cows are yielding but little milk the intervals are less equal.

The distribution of calving dates is shown in Table I.

Table I.

	No. of cows which calved during the month												
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1st calvers	3	3	3	5	2	2	1	2	—	5	5	9	40
2nd calvers	4	1	6	2	4	—	1	1	2	3	5	4	33
3 or more calves	1	2	4	7	4	3	2	2	1	4	5	3	38
All cows	8	6	13	14	10	5	4	5	3	12	15	16	111

It will be seen that a certain number of calvings occur in every month of the year although the distribution is not even, the lowest number of cows calving during the summer months and the greatest number from October to December.

PART I.

The records analysed in Part I of this work were 97 lactation records obtained from Shorthorn cows.

Lactation curves of milk yields.

In order to prepare curves showing the average yield of milk throughout a lactation period, all cows for which complete lactation records were available were classified and an average yield obtained for each month subsequent to calving; curves were thus obtained showing the average yield of milk throughout a lactation period—the records were not carried beyond the eighth month owing to a proportion of short lactation records.

The morning and evening yields were treated separately and the curves during the first eight months of the lactation period are shown in Chart 1.

The shape of the lactation curve for daily milk yield is comparatively

well known and need not be discussed at length, the usual form of curve reaches a maximum daily yield about 50 days after calving and decreases thereafter. In this paper, however, separate curves have been prepared for morning and evening yields, and Chart 1 shows that there is little difference between the behaviour of the two curves. Attention is drawn to the figures at the foot of Chart 1 showing the proportional yields of milk at a morning milking when the evening yield is unity, these figures indicate that in late lactation the morning yields are proportionately higher than in early lactation.

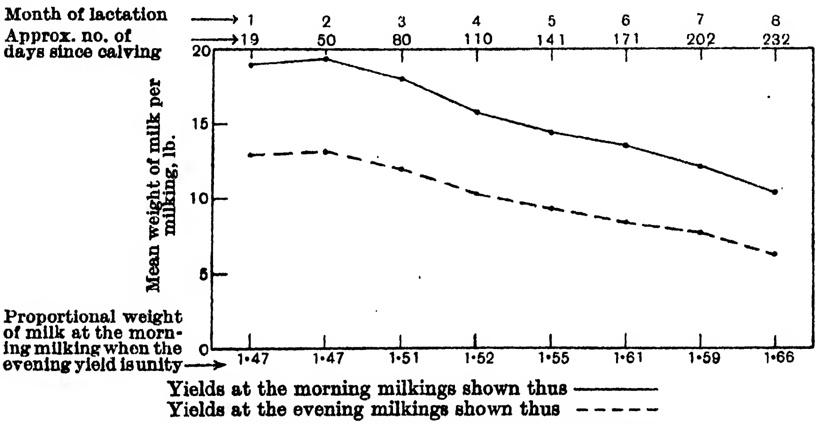


Chart 1. Showing the mean milk yields of 97 cows during each month of lactation.

The intervals between milkings average 8¾ hours and 15¼ hours, a ratio of 1 : 1·74, so that the rate of milk secretion is slightly slower during the long interval. Part of the reason for a proportionately higher morning yield during the latter part of the lactation may be found in the intervals between milkings which tend to be more uneven when the cows are yielding less milk, but this factor can scarcely account for all the difference, 1 : 1·47 in early lactation and 1 : 1·66 in late lactation.

Lactation curves of fat yields.

The weight of fat produced at each milking was calculated from the weight of milk and the percentage of fat, then by a similar classification to that used for milk yields, lactation curves were prepared for morning and evening yields of fat during each month of lactation. The results are shown in Chart 2. It will be observed that the yield of fat during a lactation period produces a curve which approximates to a straight line

indicating that the rate of decrease in the production of fat is fairly constant throughout the lactation period. On comparing the curves for morning and evening yields it will be observed that in early lactation the weights of fat yielded at the morning and evening milkings are practically the same, but as the lactation period progresses the difference between morning and evening yields becomes more and more pronounced; this point will be seen quite clearly by noting the figures at the foot of Chart 2 which express the morning yields of fat as a proportion of the evening yields during each month of lactation.

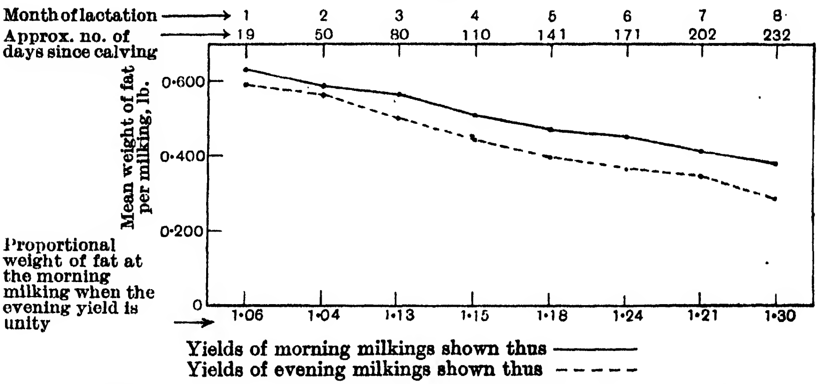


Chart 2. Showing the mean yields of fat of 97 cows during each month of lactation.

In order to obtain the average percentage of fat during each month the total weight of fat was divided by $\frac{\text{total weight of milk}}{100}$ and the results are shown in Chart 3. It will be observed that from the fourth to the eighth month of lactation the evening milk contained about 1 per cent. more fat than the morning milk, whereas during the first three months of lactation the morning milk appears to be subject to a still greater depression in butter fat percentage.

Factors influencing the lactation curves of milk and fat yields.

(1) *Age of cow.* In order to ascertain the differences in lactation curves of cows of different ages the lactation records were divided into three groups:

(*a*) First calvers.
(*b*) Second calvers.
(*c*) Older than second calvers.

Table II gives the mean monthly yields for each of the three "Age" groups of cows in respect of milk yield and fat yield.

The comparative behaviour of the milk yields at morning and evening milkings shows no important differences in the three age groups.

With regard to yield of fat, however, a striking difference is found. First calvers in early lactation produce approximately the same or even a greater weight of fat at the evening milking than they do at the morning milking, and this condition gradually disappears with the advance of lactation. The second calvers produce a little more fat at the morning milking, and in the case of the older cows the morning fat yield

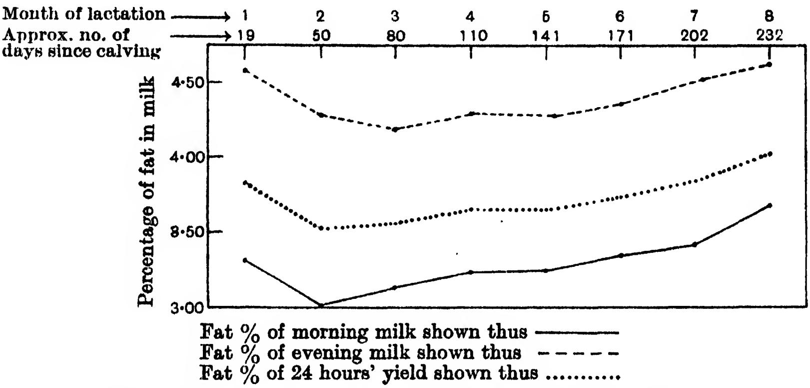


Chart 3. Showing the mean percentage of fat in the milk of 97 cows during each month of lactation.

is proportionately greater; in all age groups, however, the proportional yield of fat at the morning milking is greater in the later stages of lactation.

Comparison between the morning and evening yields may be made in another manner, viz. if it is assumed that the milk yield at the evening milking, divided by the interval since the morning milking, represents the rate of milk secretion during the first nine hours after a milking; and the additional milk obtained at the subsequent morning milking, divided by the extra interval, that which is secreted between the ninth and fifteenth hours; it will be found that during early lactation, when yields are high, the rate of secretion is comparatively low from the ninth to the fifteenth hours, whereas in late lactation the secretion from the ninth to the fifteenth hours is nearly as rapid as during the first nine hours after milking. This condition applies to yield of fat to a much greater extent than to milk yield.

Table II. *Showing the mean yield of* (a) *milk, and* (b) *fat per milking from cows of different ages during each month of lactation.*

	No. of lactations averaged		Month of lactation 1st	2nd	3rd	4th	5th	6th	7th	8th	Mean yield per milking throughout the lactation lb.
(*a*) **Weight of milk in lb.**											
1st calvers	33	a.m.	13·83	14·83	14·05	12·70	11·72	10·97	10·52	9·36	12·25
		p.m.	9·31	9·69	8·91	7·87	7·27	6·69	6·22	5·20	7·64
2nd calvers	28	a.m.	18·76	19·66	18·17	15·85	14·30	13·52	11·90	10·00	15·27
		p.m.	13·29	12·88	11·80	10·27	9·44	8·39	7·65	6·12	9·98
3 or more calves	36	a.m.	23·77	23·34	21·56	18·54	17·08	15·98	13·75	11·46	18·18
		p.m.	15·92	16·61	14·90	12·65	11·04	9·97	8·87	7·18	12·14
(*b*) **Weight of fat in lb.**											
1st calvers	33	a.m.	0·445	0·437	0·443	0·410	0·391	0·372	0·372	0·356	0·403
		p.m.	0·448	0·437	0·386	0·334	0·308	0·292	0·283	0·238	0·341
2nd calvers	28	a.m.	0·615	0·596	0·576	0·523	0·468	0·447	0·405	0·368	0·500
		p.m.	0·597	0·533	0·497	0·445	0·401	0·368	0·348	0·290	0·435
3 or more calves	36	a.m.	0·802	0·709	0·668	0·592	0·542	0·535	0·461	0·408	0·590
		p.m.	0·716	0·703	0·610	0·546	0·475	0·431	0·397	0·326	0·526

Table III. *Showing the mean yield of* (a) *milk, and* (b) *fat per milking from cows of different yielding capacity during each month of lactation.*

	No. of lactations averaged		Month of lactation 1st	2nd	3rd	4th	5th	6th	7th	8th	Mean yield per milking throughout the lactation lb.
(*a*) **Weight of milk in lb.**											
Low yielders	32	a.m.	15·34	15·07	13·75	12·60	11·83	11·41	10·03	8·39	12·30
		p.m.	10·27	9·76	8·60	7·78	7·36	6·89	6·32	5·30	7·79
Medium yielders	35	a.m.	18·66	19·86	18·18	15·65	15·10	13·89	12·39	10·52	15·53
		p.m.	12·25	12·95	12·01	10·34	9·32	8·62	7·89	6·25	9·95
High yielders	30	a.m.	23·10	23·42	22·41	19·32	16·49	15·47	14·03	12·16	18·30
		p.m.	16·50	17·11	15·50	13·06	11·34	9·76	8·67	7·10	12·38
(*b*) **Weight of fat in lb.**											
Low yielders	32	a.m.	0·566	0·507	0·465	0·436	0·407	0·395	0·347	0·322	0·430
		p.m.	0·506	0·441	0·379	0·346	0·312	0·298	0·288	0·242	0·351
Medium yielders	35	a.m.	0·595	0·596	0·569	0·522	0·475	0·469	0·414	0·378	0·502
		p.m.	0·549	0·550	0·501	0·453	0·402	0·378	0·351	0·287	0·424
High yielders	30	a.m.	0·728	0·652	0·667	0·576	0·529	0·500	0·487	0·439	0·572
		p.m.	0·730	0·710	0·632	0·539	0·482	0·424	0·395	0·331	0·531

(2) *Milking capacity.* A further classification of the lactation records was made in order to find whether the lactation curves of heavy and poor milking cows behaved in a similar manner. Each of the three groups was divided into sub-classes according to the milk producing capacity of the cows.

(i) A group of low yielding cows was selected from:

(*a*) One-third of the first calvers which were the poorest milkers.

(*b*) One-third of the second calvers which were the poorest milkers.

(*c*) One-third of the older cows which were the poorest milkers.

(ii) A group of medium yielding cows was selected in a similar manner.

(iii) A group of high yielding cows was selected in a similar manner.

The system of deciding the milking capacity was by reference to the average daily milk yield during the first three or four months of lactation, so that correction for length of lactation was unnecessary and the influence of age was avoided by dividing the cows in each age group as already mentioned. Very little difficulty was encountered in deciding in which group most of the animals were to be placed and where doubt existed the question was decided after reference to the time of year of calving and the detailed history of each animal.

The result of this grouping of animals according to milking capacity is shown in Table III; section (*a*) deals with milk yield and section (*b*) with yield of fat.

The milk yields of the different groups show no pronounced characteristics, but the yields of fat show similar tendencies to those noted in the case of the age groups. Heavy milking cows during the first two months of lactation yield a greater weight of fat at the evening than at the morning milkings, and in this respect may be compared with cows in their first lactation.

(3) *Effect of apparent high udder pressure on the lactation curve.* In view of the fact that a notable depression in yield of fat at the morning milking has been shown to be common to heifers and to high yielding animals in early lactation, it was considered probable that the condition causing this result was a high rate of secretion in comparison with the size of the milk glands. This idea was tested by asking the cowman to choose from the herd four mature cows of good milking capacity, two of which he considered developed very tight udders before the morning milking and two in which the udders never appeared to be fully distended. A comparison between the cows with these distinct types of udders is shown in Table IV. The figures are the average yields of milk and fat at morning and evening milkings during the first three months of lactation. From this table it will be seen that the cows with high udder pressure yielded a lower weight of fat at the morning than at the evening milkings. This question may be viewed from another angle—as a result of some recent unpublished work with the same herd, the writer concluded that the udders of the cows

under consideration contain about the same amount of milk and fat just before the evening milking as they do 8¾ hours after the evening milking. It is found, however, that by the usual time of the morning milking the udders contain less fat than the assumed amount about six hours previously. On this basis it seems reasonable to suggest that not only does excessive pressure of milk in the udder cause a decrease in secretion, but also induces reabsorption of part of the milk.

Table IV.

	Two cows with high pressure of milk in udders		Two cows with low pressure of milk in udders	
Milking	Milk (lb.)	Fat (lb.)	Milk (lb.)	Fat (lb.)
a.m.	25·1	0·720	19·6	0·668
p.m.	18·5	0·735	11·7	0·500

This point is not claimed as a discovery, but it is of interest to find apparent reabsorption occurring under ordinary farm conditions. The whole question has an important bearing on work dealing with the effect of age and milking capacity on fat percentage, on problems dealing with thrice daily milking and on some of the fundamentals of milk secretion, it is hoped to publish some further work on the subject in the near future.

PART II.

Yield of milk and fat at different seasons of the year.

In order to study seasonal variations in yield of milk and fat, fourteen lactation records from Guernsey cows were included with those already treated, making a total of 111 lactation records. These records were classified in twelve groups according to the month in which the lactation commenced and mean monthly yields for each of the groups were prepared. The mean daily yield of a group for a whole lactation was then ascertained and the difference between this mean and the actual daily yield for each month was expressed as a plus or minus quantity, thus the lactations starting in January provided a set of figures for each of the eight months of the year from January to August. Similarly, the lactations starting in February provided figures for February to September inclusive—and so on till each month of the year possessed a set of eight figures. By adding these plus and minus quantities for each month and dividing by eight a yield was ascertained which represented the extent to which each month's production varied above or below the normal, and by adding the results to the mean yield for the year a

representative yield for each month was obtained. The object of these calculations was to eliminate the influence of stage of lactation of the cows from the "production values" of each month, and the result indicates the monthly yield of milk which may be expected from a herd in which all cows are equally good milk producers and exactly one-twelfth of the herd calves during each of the twelve months—a set of conditions never found in practice. The lowest number of daily milk yields utilised for any monthly figure was 180, obtained from 60 separate lactation records.

Table V. *Showing mean yields per milking of milk and fat during each month of the year.*

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Mean for 12 months lb.
Milk yield (lb.)													
a.m.	14·13	14·75	15·40	16·00	17·11	16·69	14·67	15·24	14·65	14·16	13·97	14·12	15·07
p.m.	8·95	9·50	9·73	9·94	11·69	11·25	9·86	9·94	9·74	9·10	8·76	8·48	9·75
Fat yield (lb.)													
a.m.	0·485	0·496	0·519	0·520	0·550	0·534	0·502	0·492	0·512	0·488	0·450	0·485	0·503
p.m.	0·408	0·424	0·444	0·436	0·508	0·501	0·457	0·435	0·444	0·424	0·388	0·394	0·439
Fat %													
a.m.	3·48	3·46	3·46	3·41	3·33	3·34	3·50	3·38	3·51	3·50	3·38	3·59	3·446
p.m.	4·62	4·59	4·69	4·46	4·42	4·59	4·73	4·47	4·66	4·71	4·45	4·66	4·586

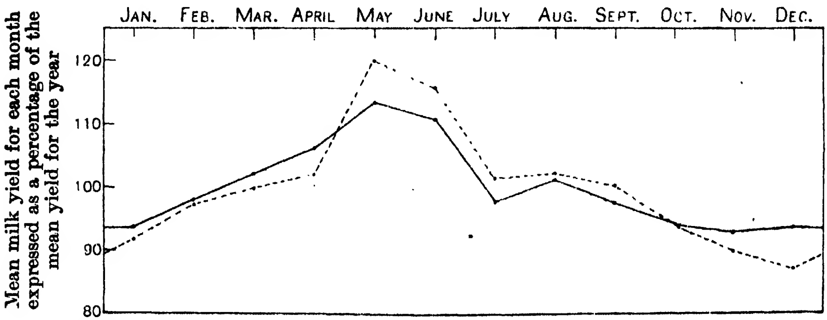
Table V shows the results for each month in respect of milk yield, fat yield and fat percentage. Chart 4 gives the monthly milk yields when expressed as a percentage of the mean yield for the year, and Chart 5 deals with similar data in respect of yield of fat.

The curves on Charts 4 and 5 show that November and December are the months of lowest production, while May and June give the highest production. Sanders (1) has calculated similar production values in respect of milk yield, and his results when expressed by the same method as that adopted for Chart 4 reach a maximum of about 117 in May and a minimum of about 89 in November, the very close agreement between the two results obtained from entirely different data suggests that both sets of figures are typical for English conditions.

Attention has been drawn to the relative position of the curves for morning and evening yields in Chart 4. It will be observed that the evening yields attain higher maximum and lower minimum values than the morning yields. It is suggested that this peculiarity is caused by the morning milking showing much less response to the milk secretion stimulus which operates during May and June.

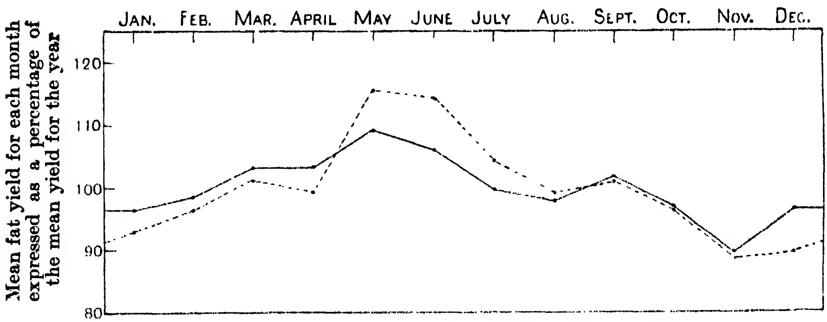
The yield of fat throughout the year (Chart 5) gives a similar curve

to the milk yield (Chart 4), the chief differences being that the maximum figures are lower and the characteristic difference between morning and evening yields even more pronounced than in the case of milk yield. This failure of the fat yield to reach the same maximum figures in May and June as the milk yield means a slight depression in the fat percentage of the milk during this period of high production.



Morning yields shown thus ———— Evening yields shown thus - - - - -

Chart 4. Showing comparative morning and evening milk yields of cows during each month of the year.



Morning yields shown thus ———— Evening yields shown thus - - - - -

Chart 5. Showing comparative morning and evening yields of fat from cows during each month of the year.

Low proportional yields of milk fat at the morning milkings in early lactation have already been noted in Part I. The practical application of this observation would seem to be that if cows are stimulated to secrete more milk, the stimulation should be accompanied by a shortening of the long milking interval in order to obtain the full benefit and to avoid a depression in the fat percentage of the milk.

The percentages of fat from month to month as shown in Table V

are remarkably constant throughout the year, and although there is a depression during May this depression is not nearly as pronounced as is usually imagined, the actual fat percentage for May being about 0·15 per cent. below the mean for the complete year.

Since the food of the cow varies considerably during the year, this curve showing seasonal variation provides some information on the effect of foods on fat percentage; and the small differences at various seasons of the year support most of the experimental evidence which has been published showing that foods do not affect the percentage of fat in milk to any appreciable extent, unless those foods upset the health or digestion of the cows.

It cannot be denied, however, that on many farms there is often trouble with poor quality milk during May and June, and it is suggested that the following represents an analysis of the factors causing this:

(1) Certain factors, the most important of which is probably young grass, increase secretion of milk at this season, and since the response to the secreting stimulus in early May is less pronounced on yield of fat than on yield of milk, the result is a slight depression in fat percentage.

(2) There is a tendency on many farms to calve a large proportion of cows during the late winter, many of these cows reach the stage of lactation when the rate of milk secretion is at its maximum and the fat percentage at its minimum during May and June.

(3) The high rate of secretion in the case of heifers and cows yielding large quantities of milk causes excessive udder pressure at the morning milking, and this adds to the depression in fat percentage at this milking.

(4) The greater variability of fat percentage from day to day during May results in occasional samples of poor quality, even if the average quality be the same as in other months.

In herds where all these factors operate in favour of low quality milk during May and June the effect may be considerable, a fact which may be observed from the records of most milk factories and collecting depots where bulk samples of milk are tested from herds throughout the year.

The variation in fat percentage with season of the year as found in this data does not corroborate Tocher's work (2), but it would appear probable that his results are influenced by stage of lactation and possibly other factors which might be classed under the heading of management, so that similar results are not to be expected.

SUMMARY.

1. Material is presented which shows month by month the lactation yields of cows in respect of milk and fat. Morning and evening yields are treated separately and differences in relative proportions found.

2. Smaller proportions of milk and of fat at the morning milkings are yielded in early lactation by all cows, but this point is most pronounced in heifers and also in heavy yielding cows with relatively small udders. It is suggested that with such animals reabsorption of milk occurs during a long night interval.

3. Seasonal variations in yield of milk and fat are shown. It is found that the morning milking does not respond as much as the evening milking to the stimulus to secretion which functions during May and June.

4. The quality of milk at different seasons of the year is discussed.

In conclusion I should like to record my thanks to Dr Stenhouse Williams and Mr James Mackintosh for considerable help and constructive criticism during the course of this work.

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THE "INOCULATION" OF LUCERNE (*MEDICAGO SATIVA*, L.) IN GREAT BRITAIN.

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(With Four Text-figures.)

1. Introduction.

The discovery in 1884–1886 that the fixation of nitrogen by leguminous crops was due to the infection of their roots by certain bacteria, quickly led to attempts to supply the crop with the necessary organisms. The earlier trials were made at Bremen Experimental Station in 1887 and two years later by Atwater and Woods in Connecticut (1). They consisted in treating the ground sown with a particular legume crop by spreading old soil from a field on which the same crop had been grown. This method has since become a recognised practice in many places with certain legume crops. Considerable quantities of soil, say from 300 to 500 pounds per acre, are needed to ensure successful infection (2), so that the expense of transportation becomes prohibitive where a crop is being introduced into a new district. Moreover there is some danger of introducing plant diseases with the soil. These considerations led to the idea of treating the seed with pure cultures of the nodule bacteria, a process that has come to be known as "inoculation." The earliest experiments with pure cultures were made in Germany by Nobbe in 1896 (3) and in Alabama, U.S.A., by Duggar (4) in 1898. Although the experiments were at first hopeful, very variable results were obtained by later workers. An attempt was made to introduce legume "inoculation" into farming practice before the life-history of the organism and its relation to the host plant had been investigated, and while the methods for the preparation and application of pure cultures to the seed were still very inadequate.

It is now known that the nodule bacteria can be divided into a number of physiological groups each of which can normally infect only a small group of legume species (5). The nodule organisms will survive for a great many years in suitable soil (6), and consequently a legume crop that is either indigenous or has been frequently grown in a district will usually find the soil already infected with its own variety of the organism. On the other hand a recently introduced legume crop will

probably find its own specific variety of the bacteria absent from the soil, whilst other varieties cannot normally produce nodules upon it. It is therefore in the case of legume crops introduced into new districts that inoculation is mainly required, and it is with such crops that most successful results have been obtained.

By far the most important of such crops is Lucerne (*Medicago sativa*), the cultivation of which has spread over large areas in all parts of the world during the last hundred years. Indigenous to Western Asia, lucerne was introduced into Southern Europe in classical times and its cultivation gradually spread northwards, reaching England in the middle of the seventeenth century. The seed was on sale in London in 1651 (7). Since then the cultivation of the crop has been mainly confined to the south-eastern quarter of England. The soil in this region has by this time become infected with the lucerne variety of the nodule organism. Commercial samples of lucerne seed are frequently contaminated with the organism. Thus it has been found at Rothamsted that when a sample of lucerne seed is sown in sterilised sand, the resulting seedlings develop a small number of nodules although under similar circumstances no nodules appear if the outer surface of the seeds is sterilised with mercury chloride. It is thus probable that the establishment of the lucerne organism in the soil of the south-eastern counties has been gradually brought about by its chance introduction in samples of the seed.

In the remaining districts of Great Britain, where lucerne is only very occasionally grown, it frequently fails and in many such cases no nodules are developed. There seemed a possibility, therefore, that a dominant cause of failure in these districts was the absence of the lucerne nodule organism from the soil, the number of bacteria introduced by chance with the seed being insufficient to produce any result in a single sowing. If this were so it seemed that the area of successful lucerne cultivation might be extended by introducing the organisms with the seed.

Various attempts to improve lucerne growth by inoculation had been made in past years. Those at Rothamsted, where the soil already contains the lucerne organism, did not give beneficial results. A success, however, was obtained by Wright (8) at the West of Scotland Agricultural College in an experiment commenced in 1905 and carried on for five years.

Of recent years our increasing knowledge of the nodule organism has led to considerable improvements in the technique of inoculation. It is now known (9) that the nodule bacteria infecting lucerne are divisible into distinct groups which differ in their nitrogen fixing efficiency and can also be distinguished by serological tests and by their growth on

media. It is therefore important that an efficient variety be used. The strain employed in the field trials described below belongs to the more efficient of these groups and was obtained from the Danish State Laboratory, Lyngby, Copenhagen. The strain was compared by Cunningham (10) with one obtained from the United States Department of Agriculture. In a pot experiment, lucerne inoculated with it showed an increase in air dried crop of 81·5 per cent. over the uninoculated, while the American strain produced an increase of 16·3 per cent., both after 3 months' growth. In the earlier work on inoculation a frequent cause of failure was the death or loss of virulence of bacteria in the laboratory. This can now be overcome by storing cultures of the organisms in sterilised soil, a method suggested by Simon (11). For the preparation of the cultures for issue to the farmers, on the other hand, a medium is needed upon which rapid, vigorous growth will be induced so that fresh cultures can be produced at as short notice as possible. P. H. H. Gray at Rothamsted has produced an agar medium containing an extract of lucerne roots upon which abundant growth can be obtained after four days' incubation. Finally, recent knowledge of the life-cycle of the nodule organisms in the soil has resulted in the development of a method for applying them to the seed, that results in the production of motile forms in the soil, thus increasing the chances of root infection. This method, developed by Thornton and Gangulee (12), has been used throughout the field trials described below.

The better chances of success with inoculation due to increased knowledge and to improvements in technique, therefore, made it worth while to give the process a critical test in this country. For this purpose the lucerne crop was particularly suitable, since it was possible to test inoculation in the soils of the south-eastern counties where the lucerne organism was already established, and in other districts where the organism was thought to be generally absent from the soil. Since it was in the latter districts that beneficial results from inoculation were to be expected, the problem was essentially bound up with the geographical distribution of the lucerne organism in British soils. The information obtainable from isolated experiments could not therefore be applied to other areas, since it was probable that inoculation would be beneficial in some districts and not in others. It was necessary therefore to make trials in a large number of localities in order to see in what districts inoculation would be effective. There was also the hope that new areas might be found where, by means of inoculation, the successful growth of this valuable forage crop might be possible. Through the generosity

of the Royal Agricultural Society in providing grants of money an extensive series of experiments in localities covering the greater part of Great Britain have been organised during the past five years. These experiments have been carried out at Agricultural Colleges, Farm Institutes and especially by private landowners, often under the supervision of the local agricultural officer. Careful experiments from so many widely scattered localities were rendered possible only by the keen and generous cooperation of these many experimenters, who have spared neither the trouble nor the expense involved and to whom the successful results are largely due.

2. PLAN OF THE EXPERIMENTS.

In order to make the results from different localities comparable the experiments were carried out as far as possible on the following general plan. Eleven plots usually of 1/5th acre were laid down, six being sown with untreated and five, arranged alternatively with them, with inoculated seed. The plots in the earlier trials were separated by 2–3 foot paths, but these were later omitted on account of the difficulty in keeping them weeded. The sowing and subsequent operations on the plots were performed on the untreated plots first, in order to lessen their accidental infection with inoculated soil. The time of sowing was necessarily determined by local circumstances. In 1926 a number of experiments were laid down with the object of comparing the spring and summer sown lucerne, as well as the effect of inoculation. These trials which are described below consist of 12 plots, half of which were sown with inoculated and half with untreated seed. In addition to the above experiments, several smaller trials have been made in various places.

The cultures used for inoculation were grown in test tubes on slopes of the lucerne root-extract agar medium previously mentioned. The cultures were incubated at 25° C. for 4 to 7 days before issue to the experimenter and were used within 6 weeks of their preparation. In treating the seed the method of Thornton and Gangulee (12) was employed. The bacteria were applied at the rate of 1 culture to 7 lb. of seed. Experiments now in progress suggest that this quantity of seed can be greatly increased with little loss of effect. The cultures were suspended in a quantity of skim milk that would wet the whole mass of seed. It was found that ¼ pint to 7 lb. of seed would just do this. Before adding the culture, calcium di-acid phosphate, $CaH_4(PO_4)_2 + 2H_2O$, was dissolved in the milk to make a 0·1 per cent. solution, the correct amount of this salt being sent out with the cultures. The milk containing the

Table I. *List of experiments to test lucerne inoculation.*

No. on map	Experimenter	Trial under the supervision of	Soil type	Date of sowing	Cover crop	No. of plots	Size of plots (acre)
	A. *Western area:*						
1	F. Ballard, Maybole, Colwall, Hereford	J. Ll. Evans	Light limestone	May 1926	No	4	¼ to 1
2	R. T. Board, Merthyr Mawr, Bridgend, Glamorgan	J. D. Davidson and H. Rhys Williams	Light gravel	May 1924	No	11	1/5th
3	R. V. Bradburn, Stanmore Farm, Bridgnorth	—	Light	May 1924	No	11	1/5th
4	Col. E. P. Brassey, Upper Slaughter, Gloucestershire	G. H. Hollingworth and C. Comely	Shallow limestone	May 1925	Yes	11	1/5th
5	A. T. Cake, Higher Came, Dorset	T. R. Ferris and J. A. Robotham	Light chalk loam	May 1926	No	11	1/5th
6	Rt Hon. Lord Clinton, Rolle Estate, Exmouth	—	Red sandstone	June 1925	No	4	½
7	G. H. Johnstone, Tregoose, Grampound Road, Cornwall	A. Gregg	Clay shale	May 1925	No	11	¼
8	J. H. Malcom, Ty Gwyn, Clydack, Swansea	J. D. Davidson and H. Rhys Williams	Poor grit soil	May 1924	No	11	1/5th
9	A. S. Matthias, Llangwarren, Pembrokeshire	—	Shale	April 1925	Yes	4	¼
10	Seale Hayne Agric. College, Newton Abbot	T. J. Shaw	Shallow shale	April 1924	Yes	11	1/5th
11	G. Sheaf, Honeybourne, Gloucestershire	G. H. Hollingworth and C. Comely	Heavy clay	May 1925	No	11	1/5th
12	W. Smith, Cogan Hall, Penarth	J. D. Davidson and H. Rhys Williams	Clay loam	June 1924	No	11	1/5th
13	Welsh Plant Breeding Station, Aberystwyth	Prof. R. G. Stapledon	Poor shallow shale	May 1925	No	21	1/24th
	B. *Northern area:*						
14	J. D. Johnstone, Eden Lacey, Lazonby	Principal R. Lindsay Robb	Sandy loam	June 1926	No	11	1/5th
15	W. Low, Balmakewan, Marykirk	Prof. J. Hendrick	Red sandstone	May 1924	No	11	1/5th
16	J. W. McGillivray, Aberdeen	—	Loam over clay	May 1927	No	4	1/16th
17	Pennel and Sons, Welton, Lincoln	—	Clay	June 1926	No	8	1/8th
18	E. Abel Smith, Longhills, Lincoln	—	Gravel	April 1924	No	11	1/5th
19	W. R. Strickland, Bainess, Catterick	—	Clay	June 1925	No	10	1/10th
20	J. Walker, Houghall, Durham	—	Gravel	June 1925	No	8	1/8th

	C. *Central area:*						
21	A. T. Carr, Turner's Court, Wallingford	—	Chalk	Aug. 1925	No	11	1/5th
22	C. C. Edmunds, Mentmore Estate, Leighton Buzzard	—	Heavy clay	April 1924	No	11	1/5th
23	C. Barwell Field, Bowmans, Colney, Herts.	—	Gravel loam	Aug. 1924	No	11	1/5th
24	Herts. Institute of Agriculture, Oaklands, St Albans	J. Hunter Smith and H. Rhys Williams	Clay with flints	April 1925	Yes No	10 11	½ 1/5th
25	W. Keevil, Berhill, Calne, Wiltshire	—	Oolite limestone	May 1924	No	11	1/5th
26	W. Lawson, West Sussex County Council Farm, Chichester	—	Clay	May 1926	No	11	1/10th
27	Col. C. Lyon, Appleton Hall, Cheshire	W. B. Mercer	Clay loam	July 1925	No	6	1/10th
28	Col. F. H. N. Meynell, Hoar Cross, Burton-on-Trent	—	Gravel	June 1925	No	6	1/6th
29	G. W. Olive, Dauntsey School, Wiltshire	—	Sandy loam	April 1924	No	8	1/12th
30	Studley College, Warwick	Miss Melville Jackson	Loam	July 1925	No	11	1/10th
31	Woburn Experimental Station	Dr J. A. Voelcker	Sandy	July 1927	No	11	1/5th
32	A. A. White, Ardley Fields, Bicester	G. R. Bland	Limestone	July 1926	No	10	1/5th
	D. *South-eastern area:*						
33	Australian Farms Training College, Lynford Hall, Norfolk	Principal H. H. Potts	Sandy loam	Sept. 1926	No	11	1/5th
34	A. E. Meeson, Eastling, Faversham	—	Clay with flints	Aug. 1924	No	11	1/5th
35	A. W. Oldershaw, Tunstall Heath, Suffolk	—	Light sand	July 1926	No	6	1
	Spring and summer sown trials:						
36	A. Clarke and Sons, Chiselborough, Somerset	W. D. Hay and J. D. Dallas	Sandy	May and July 1926	Spring sowing with cover crop	12	1/10th
37	County School, Welshpool, Montgomery	C. Harrison	Shale	—	„	12	1/40th
38	C. H. Roberts, Boothby, Brampton	H. W. Cousins	Sandy	April and July 1926	„	12	1/8th
39	Rothamsted Experimental Station	—	Clay with flints	April and July 1926	„	12	1/10th

cultures in suspension was poured on to the seed and thoroughly mixed with it, until every seed was wetted. The seed was then spread out in the shade and allowed to dry. It was found to be very important that the seed should be completely dried, otherwise difficulty was experienced owing to the seed clogging the drill. The use of skim instead of whole milk greatly shortens the time of drying.

Since little was known as to the suitability of many districts for growing inoculated lucerne it was inevitable that at a number of centres the crop should fail. The causes of crop failure are discussed below, but in discussing the effects of inoculation only those trials are considered which are still running or which were continued for long enough for the effect of the inoculation to be determined. A list of these centres is given in Table I.

3. Effect of Treatment in Different Parts of Great Britain.

The map, Fig. 1, shows the distribution of the trials that are here discussed, indicating in which of them an improved growth as a result of inoculation has been observed.

A summary of the yield and analysis results so far obtained from the trials is shown in Tables II, III and IV. Wherever possible the whole crop from each plot was separately weighed, but in some cases, as shown in the table, a cutting from an equal area on each plot was taken. The figures give mean weights and analyses from the parallel plots.

There is no evidence that the response to inoculation is related in any way to soil or to climatic conditions. As was expected, however, the effects produced by inoculation do vary in different districts. It is thus convenient to divide the country into areas and to consider the experiments in each area separately.

A. *The south-western area.* This includes Wales and the counties of Shropshire, Hereford, Gloucester, Somerset, Dorset, Devon, and Cornwall. A marked benefit from inoculation has been observed in almost all experiments in this area (Table II). The effect has usually shown itself quite early in the life of the seedling plant and has persisted throughout the trial save where there has been evidence that the untreated plots have become infected. In Wales, Mr Harrison obtained a 58·4 per cent. increase in crop from inoculation, while, in a trial made by Prof. Stapledon at the Welsh Plant Breeding Station, Aberystwyth, the inoculated plots bore a stronger and deeper coloured growth during the first year. In this latter trial, however, the untreated plots became infected before weighings were taken. The experiments in Glamorgan and the small

Table II. *Yield and nitrogen contents of the crops. South-western area.*

Experimenter	Year of sowing	Month and year of cutting	Weights obtained from: No. of plots	Weights obtained from: Area per plot in sq. yds.	Whether weighed green or as hay	Yield in cwts. per acre: Inoculated	Yield in cwts. per acre: Untreated	Percentage increase over untreated	Percentage nitrogen content: Inoculated	Percentage nitrogen content: Untreated	Cwts. of nitrogen per acre in crop: Inoculated	Cwts. of nitrogen per acre in crop: Untreated
Col. E. P. Brassey, Gloucester	1925	June 1926	11	968	Hay	23·7	14·5	63·4	2·77	2·59	0·66	0·38
		Sept. 1926	11	968	Green	40·2	14·2	183·1	—	—	—	—
		June 1927	11	82	Hay	109·3	32·5	236·1	2·53	2·1	2·77	0·68
		Sept. 1927	11	67·5	Green	71·7	38·4	86·7	—	—	—	—
		June 1928	11	73	Green	91·7	62·0	47·9	2·97	2·02	2·72	1·75
		Aug. 1928	11	41	Green	49·5	33·0	52·7	—	—	—	—
A. T. Cake, Dorset	1926	June 1927	11	66	Hay	20·9	17·0	23·0	—	—	—	—
Rt Hon. Lord Clinton, Devon	1925	July 1926	11	605	Semi-dry	53·0	34·0	55·9	3·91	3·13	2·07	1·06
		July 1927	11	605	Semi-dry	52·0	50·0	4·0	3·38	3·24	1·76	1·62
G. H. Johnstone, Cornwall	1925	Sept. 1925	8	80	Green	44·25	35·25	25·5	—	—	—	—
		July 1926	4	40	Semi-dry	61·5	41·9	46·8	3·4	3·3	2·09	1·38
		Sept. 1927	9	48	Semi-dry	38·2	39·3	−2·8	4·1	3·6	1·56	1·41
G. Sheaf, Gloucestershire	1925	Sept. 1925	11	145	Green	21·8	7·8	179·4	3·94	2·84	0·86	0·22
		Aug. 1926	11	108	Green	44·7	32·5	37·5	3·34	3·3	1·49	1·07
		June 1927	11	36	Green	61·2	51·6	12·7	2·54	2·36	1·55	1·22
Welsh Plant Breeding Station, Cardigan	1925	July 1926	12	20	Green	192·7	174·2	10·6	—	—	—	—
		July 1927	12	20	Green	118·4	112·4	5·3	—	—	—	—
Clarke and Sons, Somerset	1926	Oct. 1926	6	30	Green	37·5	33·8	10·9	2·57	2·62	0·96	0·88
		July 1927	12	484	Green	81·75	66·8	22·4	4·13	4·12	3·38	2·75
C. Harrison, Montgomery	1926	July 1927	12	121	Green	87·1	55·0	58·4	2·55	2·66	2·22	1·46
		Oct. 1927	12	121	Green	43·9	33·3	31·8	2·85	2·9	1·25	0·97

trial made by Mr Matthias in Pembrokeshire all showed a visible difference from treatment. A very marked effect was also observed in Mr Braburn's trial in Shropshire and in Mr Ballard's in Herefordshire.

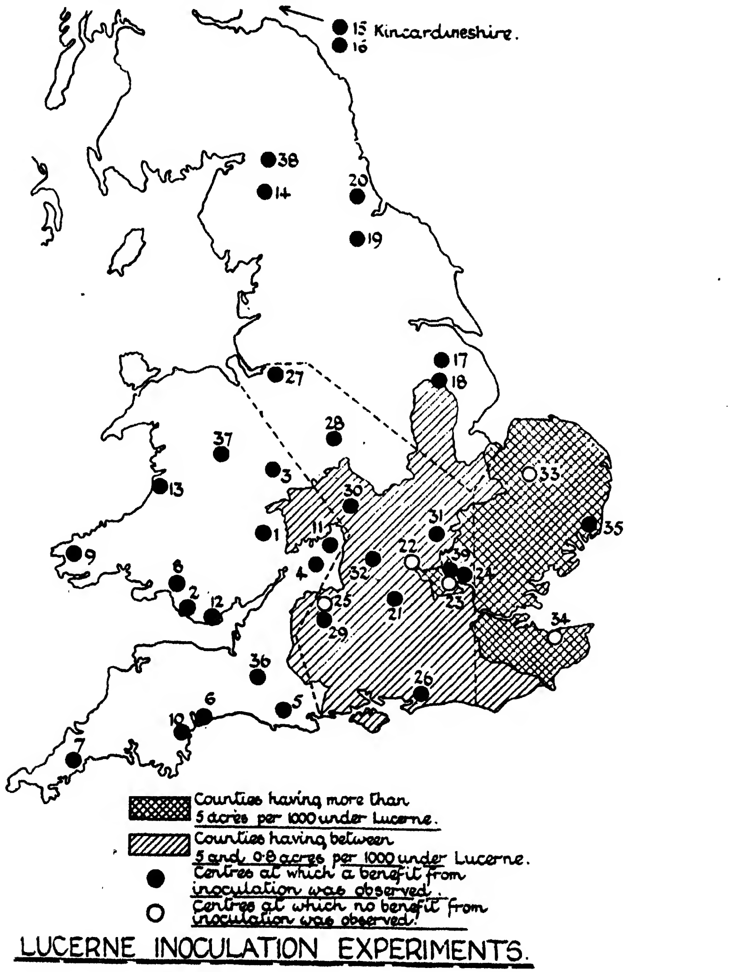


Fig. 1.

The most striking results, however, have been obtained from Gloucester, increases of 236·1 per cent. and 179·4 per cent. in the yield having been recorded from Colonel Brassey's and Mr Sheaf's experiments. Examination of the roots of the untreated plants on Colonel Brassey's land showed an almost complete absence of nodules. Thus 150 untreated

Table III. *Yields and nitrogen contents of the crops. Northern area.*

Experimenter	Year of sowing	Month and year of cutting	Weights obtained from: No. of plots	Weights obtained from: Area per plot in sq. yds.	Whether weighed green or as hay	Yield in cwts. per acre: Inoculated	Yield in cwts. per acre: Untreated	Percentage increase over untreated	Percentage nitrogen content: Inoculated	Percentage nitrogen content: Untreated	Cwts. of nitrogen per acre in crop: Inoculated	Cwts. of nitrogen per acre in crop: Untreated
W. Low, Kincardineshire	1924	Oct. 1924	22*	484	Green	83·5	75·8	10·2	—	—	—	—
		July 1925	22	484	Hay	48·3	34·3	40·9	—	—	—	—
		Sept. 1925	22	484	Green	60·3	51·3	17·5	—	—	—	—
		July 1926	22	484	Hay	41·5	37·5	10·7	—	—	—	—
		Sept. 1926	22	484	Green	63·75	61·25	4·1	—	—	—	—
		Aug. 1927	22	484	Hay	58·5	55·5	5·4	—	—	—	—
		Sept. 1927	22	484	Green	33·5	32·0	4·7	—	—	—	—
Pennell and Sons, Lincoln	1926	July 1927	8	605	Green	151·5	125·5	20·7	2·64	2·56	3·99	3·2
W. R. Strickland, Yorkshire	1925	July 1926	11	40	Green	100·0	62·1	61·0	3·4	2·2	3·4	1·37
J. Walker, Durham	1925	Sept. 1925	8	302	Green	62·5	53·5	16·8	—	—	—	—
		Sept. 1926	6	605	—	173·0	87·4	98·0	—	—	—	—

* In Mr Low's experiment each plot was divided longitudinally and the crop from each half separately weighed.

lucerne plants examined bore only 22 nodules, whereas 100 inoculated plants from adjacent plots bore 341 nodules. It is thus probable that the lucerne organism is absent from this soil, accidental transfer of bacteria in the seed sample being sufficient to account for the small number of nodules on the control plants.

In Somerset, Messrs Clarke and Sons' experiment showed an increase of 22·4 per cent. in 1927. In Devon, Lord Clinton's trial showed an increase of 55·9 per cent. in 1926, and in Cornwall, Mr Johnstone's trial showed an increase of 46·8 per cent. in the same year. At Seale Hayne Agricultural College, the inoculation produced a visible improvement, but the crop failed owing to the shallowness of the soil. In Dorset, Mr A. T. Cake obtained an increase of 23·0 per cent. from inoculation.

B. *Northern area.* In the northern half of England and in Scotland, the experiments have invariably shown a benefit from inoculation as in the south-western area (Table III). In Lincolnshire, Messrs Pennell and Sons obtained an increase of 20·7 per cent. in yield from the treatment, and much of the untreated lucerne died and was replaced by rye grass, while Mr Abel Smith's plots showed a visible effect. In Mr Strickland's experiment at Catterick the treatment increased the yield by 61 per cent., and in the experiment at Houghall Farm, Durham, by 98 per cent. At the two centres in Cumberland the inoculated plots were visibly stronger from the first, indeed, in Mr Roberts' experiment, only the inoculated lucerne grew. In Scotland, Mr Low's trial near Montrose showed an increase of 40·9 per cent. in 1925. A number of trials organised by Cunningham (10) near Edinburgh and Wright's trial (8) at the West of Scotland Agricultural College in 1908, showed that in other parts of Scotland, also, inoculation produces a striking effect.

C. *The central area.* This forms a broad zone separating the western area discussed from eastern lucerne growing area. Experiments in the counties of Cheshire, Staffordshire, Warwick, Berkshire, Hertford, Wiltshire, Hampshire, and West Sussex mostly show an increased growth from inoculation which, though sometimes considerable at a certain stage in the trial, is transient (Table IV). Table V shows in several trials how soon after sowing the effect from inoculation became visible and for how long this effect remained.

In plots sown without a cover crop, after periods varying from 6 to 14 months the growth of the untreated lucerne has caught up that of the inoculated plants. The trial at Rothamsted is typical. It is known as a result of numerous experiments that the soil at Rothamsted contains the lucerne nodule organism, but in such small numbers that

Table IV. *Yields and nitrogen contents of the crops. Central and south-eastern areas.*

Experimenter	Year of sowing	Month and year of cutting	Weights obtained from: No. of plots	Weights obtained from: Area per plot in sq. yds.	Whether weighed green or as hay	Yield in cwts. per acre: Inoculated	Yield in cwts. per acre: Untreated	Percentage increase over untreated	Percentage nitrogen content: Inoculated	Percentage nitrogen content: Untreated	Cwts. of nitrogen per acre in crop: Inoculated	Cwts. of nitrogen per acre in crop: Untreated
Central area:												
A. T. Carr, Berks.	1925	July 1926	11	30	Green	7·8	6·1	27·9	—	—	—	—
Hertfordshire Institute of Agriculture	1925	May 1926	5	968	Hay	219	167·6	30·0	—	—	—	—
		July 1926	5	968	Hay	178·5	147·8	20·8	—	—	—	—
		Sept. 1926	5	968	Hay	44·6	37·6	18·6	—	—	—	—
		May 1927	5	968	Green	37·4	34·1	9·5	—	—	—	—
		July 1927	5	968	Green	28·1	27·2	3·3	—	—	—	—
		Sept. 1927	5	968	Green	12·05	12·2	-0·8	—	—	—	—
W. Lawson, Sussex	1926	May 1927	11	968	Green	211·6	187·2	13·4	3·56	3·36	7·5	6·3
		July 1927	11	968	Green	177·5	166·5	6·6	—	—	—	—
Col. C. Lyon, Cheshire	1925	June 1926	6	242	Green	118·3	110·7	6·9	4·61	3·99	5·45	4·41
Col. F. H. N. Meynell, Staffordshire	1925	Oct. 1925	6	807	Hay	14·54	14·28	1·8	3·21	2·19	0·47	0·31
Studley College, Warwickshire	1925	Sept. 1925	11	28	—	162·8	120·4	35·2	—	—	—	—
		June 1926	11	3	—	216·9	210·4	3·1	2·93	2·88	6·36	6·06
		June 1927	11	11	—	191·3	180·4	6·0	2·65	2·64	5·07	4·76
Woburn Experimental Station, Bedfordshire	1927	July 1928	11	726	Hay	38·0	30·8	23·4	3·18	1·65	1·21	0·51
A. A. White, Oxford	1926	June 1927	11	968	Green	22·8	21·95	3·6	2·99	3·04	0·68	0·67
South-eastern area:												
Australian Farms Training College, Norfolk	1926	Aug. 1928	11	968	Hay	4·97	5·32	6·6	—	—	—	—
A. W. Oldershaw, Suffolk*	1926	June 1928	18	30	Green	21·6	12·2	77·5	—	—	—	—

* In Mr A. W. Oldershaw's trial the weights from the chalked plots alone are included.

the nodules on lucerne plants are greatly increased by inoculation. Thus in a pot experiment with Rothamsted soil the nodules per plant (means of 50 plants) were: inoculated, 118·3; untreated, 46·7; and in a second similar experiment (means of 10 plants): inoculated, 113·1; untreated, 43·8. This increase in nodule numbers appreciably affects the growth.

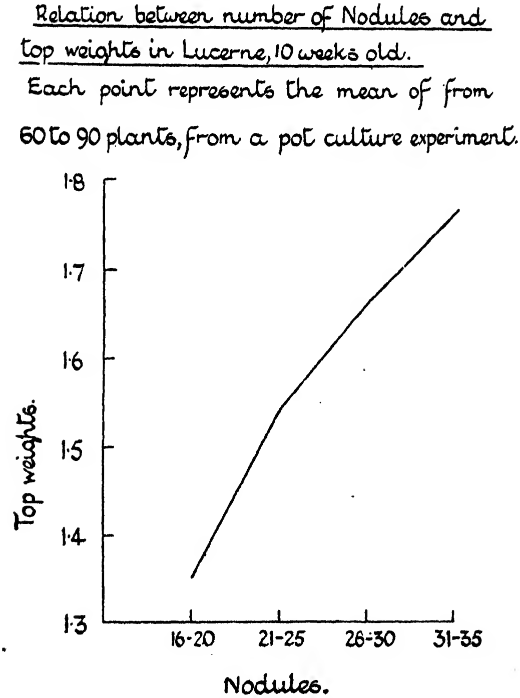


Fig. 2.

In the above pot experiments the increases in top weight of inoculated over control plants were 40·5 per cent. and 16·1 per cent. respectively. Indeed in the early period of growth the top weights and nodule numbers are correlated (Fig. 2). The heavier nodule formation produced by inoculation is thus sufficient to increase the growth of the lucerne in the first few months even in soils that contain a certain number of the lucerne nodule organisms. On clean land and under good conditions the untreated lucerne may recover from its weak start. Since the experiments mentioned in Table V show no evidence of a spread of organisms from the edges of the inoculated plots, this recovery is probably due to the multiplication of the bacteria already present in the soil. Where, however,

Table V.

Experiment	Time before the effect appeared (months)	Cover crop	How long the effect lasted (months)
Rothamsted Experimental Station	2	No	10
Studley College, Warwick	2	No	10
Oaklands Farm Institute, St Albans	2	No	6
		Yes	30
A. A. White, Bicester	9	No	10
Farm Training Colony, Turners Court, Wallingford	10	No	14
W. Lawson, Sussex	3	No	12

the young lucerne has to compete with weed infestation or with a cover crop, the inoculation may produce a much greater benefit, since, in this case, a weak young plant will tend to be smothered and, moreover, the weeds or cover crop, by competing for combined nitrogen, force the lucerne to depend upon the nitrogen from its nodules at an earlier stage of growth. The experiment at the Hertfordshire Agricultural Institute, St Albans, affords an instructive example of this. Here, the usual trial consisting of eleven plots of 1/5th acre was laid down, the lucerne being sown on bare ground in April 1925. Adjoining this were a series of plots each of approximately half an acre, in which lucerne alone and in various seeds mixtures was sown in a cover crop of barley. The arrangement of these plots was as shown in Table VI. The lucerne sown without a cover

Table VI. *Lucerne sown under a cover crop of barley at the Hertfordshire Institute of Agriculture.*

(*Trial carried out by Mr J. Hunter Smith.*)

Cutting made May 1926, 14 *months after sowing.*

Plot	Size of plots 0·75 to 1 acre	Yield in cwts. per acre: Inoculated	Untreated	Increase
A	Lucerne drilled	232·0	180·0	52·0
B	Lucerne drilled—1 lb. per acre wild white clover	233·0	217·0	16·0
C	Lucerne drilled—2 lb. per acre cocks foot ...	208·0	133·0	75·0
D	Lucerne drilled—2 lb. per acre Italian rye grass	202·0	139·0	63·0
E	Lucerne, broadcast	220·0	169·0	51·0
	Mean	219·0	167·6	51·4

crop showed a visible effect from inoculation which influenced both growth and especially the colour of the plant. This effect became noticeable in June and was very marked in August 1925. During September the untreated plots began to darken in colour uniformly, and by October it was no longer possible to distinguish between the

plots. On the plots sown in a cover crop, on the other hand, the early start due to inoculation produced a more lasting effect. In October the inoculated lucerne was about 6 inches high while scarcely any was visible on the untreated plots. In June 1926 the cover crop plots gave the yields shown in Table VI. Even on these plots, however, the untreated lucerne recovered during 1927 (Table IV). Trials made by Mr Edmunds on the Mentmore Estate, Leighton Buzzard, Mr Barwell Field near London Colney, and by Mr W. Keevil near Calne, showed no effect from inoculation. It seems probable that at these centres the soil contained a population of lucerne bacteria sufficient for the plant's needs. At Woburn Experimental Farm a marked effect from inoculation still persists. The previous acidity of the soil here makes this experiment more comparable with that at Tunstall, Suffolk, which is discussed below.

The experiments in the midlands and south central counties, taken as a whole, indicate that the soils usually contain a sparse population of lucerne bacteria which, if the crop survives long enough, will eventually infect it, but that, in these soils, inoculated lucerne makes a stronger growth in the young stages, an advantage which may produce a lasting effect on the crop where it has to compete with other plants. In this district, therefore, inoculation is advisable as a precaution against unfavourable conditions in the first year and should always be adopted when the lucerne is sown in a cover crop.

In the south-eastern counties about a dozen trials were started, the majority of which unfortunately failed owing to the wet summer of 1924, but those that survived during the seedling year did not in this time show any visible improvement from inoculation. In an experiment carried through at the Australian Farms Training College at Lynford Hall, Norfolk, the yield results showed no benefit from the treatment (see Table IV). Over most of the south-eastern area the lucerne organism is apparently present in the soil as is indicated by the success with which uninoculated seed can be grown. An interesting and important exception is afforded by the trial carried out by Mr A. W. Oldershaw, on the County Council Experimental Farm at Tunstall, near Ipswich. This farm is on an area of very light, sour land, having a reaction of about pH 5·4. The trial was made both with the object of testing the value of inoculation and to see whether lucerne could be grown on this land after liming. The experimental plots of inoculated and untreated lucerne were therefore laid across strips left unlimed and limed in various ways. Table VII shows the plan of the trial. The lucerne was sown in July 1926. By the following March no effect from inoculation was visible,

Table VII. *Plan of the experiment at the East Suffolk County Council Farm, Tunstall, showing yields in* 1928, *as cwts. per acre.*

	Control	Inoculated	Control	Inoculated	Control	Inoculated	Mean yield inoculated	Mean yield control
No lime	4·5	5	4·5	5·5	4·5	13	7·5	4·5
Crag 5 tons per acre				No weights taken				
Chalk 5 tons per acre	19	26·5	12·5	16	11·5	18·5	20·3	14·3
Chalk 10 tons per acre	11·5	23	7	18·5	10	18	19·8	9·5
Chalk 20 tons per acre	12·5	37·5	14	22·5	12·5	24	24·7	13

but only the inoculated plants bore nodules. By the middle of June a very marked effect from inoculation was visible on the limed plots and analysis showed the following nitrogen percentages in tops and roots:

	Inoculated	Control
Tops	2·55	1·31
Roots	1·63	0·88

In 1928 the first cutting gave the yields shown in Table VII. The experiment shows that even in a region such as East Anglia whose soils generally contain the lucerne organisms, they may be absent where the soil is acid. This conclusion is supported by other workers. Fred and Davenport (13) testing seven strains of the lucerne organism found that in laboratory cultures in a liquid medium an acidity greater than pH 5 prevented growth, while Bryan (14) found that they were killed by storage for 75 days in soil more acid than pH 5. Joffe (15) and Bryan (16) found that nodule development on lucerne fell off rapidly as the acidity increased from pH 7 to pH 4 although a few nodules developed at the latter reaction. The dying out of lucerne bacteria in acid soils thus makes inoculation important in these soils after they have been limed. Thus Fred and Graul (17) found that in the acid soils of Wisconsin inoculation was very beneficial especially when combined with liming. This is also the common experience in Denmark.

4. Spreading of the Bacteria.

In many cases the weight results underestimate effects of "inoculation." This is due to two causes. In the first place the untreated plots tend to become very weedy and the crop weighed often consists largely of weeds. This is for example the case on Col. Brassey's plots where, in 1927, the untreated plots bore only an occasional small plant of lucerne,

the weight of crop being made up entirely of other plants, mostly red clover (see Table II).

In the second place, there have been certain experiments where the control plots have become infected by the migration of the nodule bacteria into them from the inoculated plots. This usually shows itself above ground by the gradual spreading of the deeper green colour from the edges of the inoculated plots. In the case of Mr W. Low's and

Fig. 3.

Mr G. Sheaf's experiments the whole of the control plots have now become infected. The yields from Mr Low's plots illustrate very clearly the apparent loss of effect from inoculation which is in reality due to the gradual infection of the control plots (Fig. 3).

Observations of the spreading on Mr Sheaf's plots showed that it commenced in the middle of August 1925 and continued until winter set it, the plants in the newly infected areas bearing numerous nodules. No further spreading was noticed until the following June when the spread recommenced and continued during the summer, by the end of which the control plots were almost completely inoculated (Table II).

It is thus probable that the spreading is affected by season, and does not occur except under special conditions of soil moisture and temperature. It is at any rate an exceptional phenomenon, marked spreading having been observed in only six of the experiments. It is not apparently related to soil type, though in two cases it was clearly connected with the wetness of the soil. The migration of the bacteria must have a considerable bearing upon the slow infection of a soil by the chance introduction of small numbers of nodule bacteria in samples of uninoculated seed. The sporadic occurrence of spreading indicates that only under exceptional conditions will a soil become rapidly populated with the organism by such accidental means.

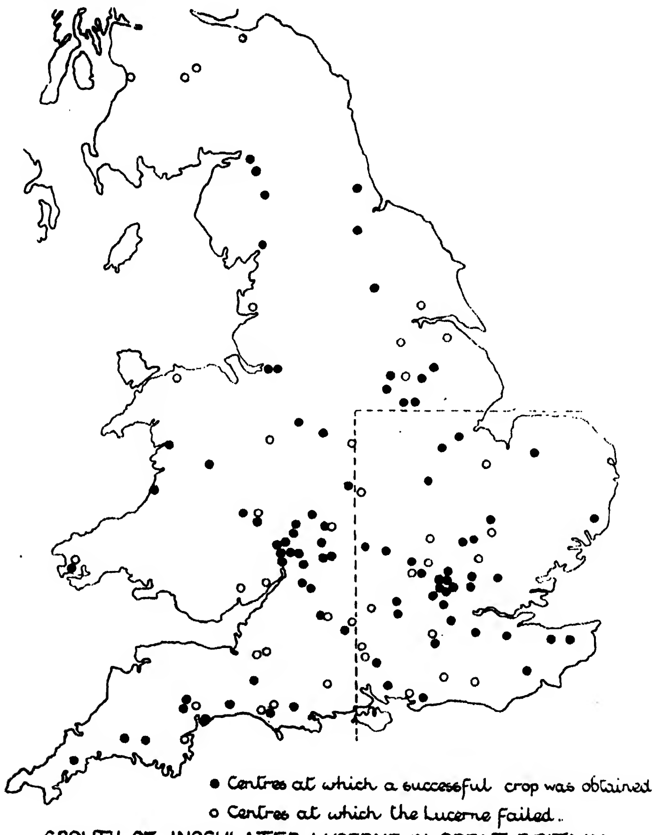
5. Nitrogen Content of the Crop.

The inoculation sometimes causes an increase in the nitrogen content of the crop without raising the yield. This has occurred, for example, in Colonel Meynell's experiment (Table IV). More often both the yield and the percentage of nitrogen are increased. In both these cases it would seem that some other limiting factor has prevented the crop from taking full advantage of the increased nitrogen supply. In other cases, as in Mr Lawson's and Messrs Pennell and Sons' trials, there is an increase in the crop but no significant increase in the percentage of nitrogen in it. In such cases the plant has been able to make full use of the nitrogen to increase its bulk.

6. Prospects of Success with Lucerne in the Various Districts.

The better crop obtained by inoculating lucerne in the south-west, west and north of Great Britain raises the question: Can the successful growth of lucerne be extended westwards and northwards by the use of the present improved methods of seed inoculation? During the last two years cultures for inoculation have been sold to about 200 farmers distributed over nearly all parts of England. The success or failure of the crop from inoculated seed has been reported in a considerable number of cases so that we now have a good deal of evidence, apart from that obtained from actual experiments as to chances of getting a crop from inoculated lucerne in various districts. The map, Fig. 4, shows the distribution of successes and failures with inoculated seed sown in 1925, 1926 and 1927. The figure only shows whether a successful crop was obtained from the inoculated seed and does not take into account the growth of untreated lucerne in places where this was sown. The area east of Longitude 1·5° and south of Latitude 53° may be taken as being

that over which the crop is at present cultivated to an appreciable extent. Within this area inoculated seed gave a successful crop at 35 out of a total of 49 centres (71·4 per cent.). In the remainder of Great Britain where the crop is at present very seldom grown, inoculated seed gave successful crops at 53 out of 78 centres (67·9 per cent.). Thus in



GROWTH OF INOCULATED LUCERNE IN GREAT BRITAIN.

Fig. 4.

spite of the fact that this latter area includes centres in such unpromising districts as Wales and Cumberland, the percentage of successes with inoculated seed is not significantly lower than in the south-eastern area. Taking the western and northern area as a whole it thus appears that the climatic and soil conditions do not prevent the cultivation of the crop when the nodule bacteria are supplied to it, but that the failure of the crop in these districts in the past has been mainly due to the absence

of the appropriate bacteria from the soil. It would especially appear that the Gloucestershire and Herefordshire district is well suited to the growth of lucerne from inoculated seed.

7. The Causes of Lucerne Failure.

The records of experiments in which the lucerne failed afford interesting evidence as to the prevalence of the various causes of failure. The trials were unfortunately started in the very wet spring and summer of 1924, and of those sown in that year 15 failed owing to weeds. The difficulty in checking the weeds in this wet year must, however, be regarded as quite abnormal. Other trials sown in this year failed for the following reasons:

Cause	No. of trials
Acid soil	3
Wet soil	2
Frost...	1
Too loose seed bed...	1
Shallow subsoil	1
Insect attack	1

The weather conditions in 1925 and 1926 were more normal. The causes of failure in trials sown in these two years were as follows:

Cause	No. of trials
Weeds	5
Drought	3
Frost...	3
Insect attack	1
Cause unknown	2

Even in these more normal years weeds were the most frequent form of failure. The three trials that failed through drought were sown in June or July, showing that there is some risk attending late sowing, although thirteen other trials sown in these two months have been successful. It is probable that soil acidity is a more common cause of failure than appears in these figures since in nearly all experiments the ground was well limed before the lucerne was sown. It seems clear, however, that the main difficulty to be met in extending the cultivation of inoculated lucerne in the west and north, is that of controlling weeds. There are three stages in the life of the plant when weed infestation seems to be especially dangerous. When the crop is sown in April or early May it usually has to compete in the early summer with spring germinating weeds. In the autumn of the seedling year there is often a heavy growth of such weeds as groundsel or chickweed which may smother the young lucerne. Finally, an old stand of lucerne is liable to be damaged by perennials and grasses. In many cases this last type of weed growth is probably a symptom of the weakening of the lucerne

plant from some other cause. It is in the seedling year that the real difficulty has to be met. It is necessary that the plant should be protected against severe weed competition until it is strong enough to smother the weeds, and therefore this period should be shortened as much as possible by inducing quick growth of the young plant. It is in doing this that inoculation would seem to be advantageous. It is the practice in some districts to sow the lucerne in a cover crop, and it is claimed that this helps to keep down the spring germinating weeds and also protects the young lucerne against adverse weather. On the other hand some successful growers of the crop sow it in late June or July. The advantage claimed for this method is, first that the spring germinating weeds can be removed before the lucerne is sown, and secondly that the warm soil induces rapid early growth of the crop. On the other hand the lucerne is exposed to the risk of drought in the young seedling stage and to the danger of severe competition with autumn weeds before it has made enough growth to smother them. It was thought advisable to make a comparison between these two methods of sowing the crop. Experiments were laid down in Somerset, Montgomery, Monmouth and Cumberland as well as at Rothamsted (see Table I), localities in the west being selected, since in such wet districts the difficulty of weed control is especially great. In each experiment the following treatments were tested in triplicate, the plots being arranged in three blocks within each of which the four differently treated strips were arranged at random:

A. Inoculated seed sown in April or May in a cover crop.
B. Untreated seed sown in April or May in a cover crop.
C. Inoculated seed sown in July on bare ground.
D. Untreated seed sown in July on bare ground.

In the experiment in Somerset laid down by Messrs Clarke and Sons in 1926 the cover crop failed. In the second year, however, the spring sown crop looked stronger than the July sown and was less weedy. In July 1927 weighings of the green crop were made and gave the results shown below:

Treatment	Yield in cwts. per acre. Mean of 3 plots	Percentage nitrogen in the crop
Spring sown, inoculated	119·3	4·13
Spring sown, untreated	97·2	4·09
July sown, inoculated	44·2	4·12
July sown, untreated	36·5	4·14

A similar trial laid down at the Agricultural Institute, Usk, failed probably owing to water logging of the soil in winter. In a trial laid down in 1926 at the County School, Welshpool, Montgomery, the plots became much infested with chickweed and groundsel in the autumn of

the seedling year. It was noticed that the spring sown plots were the better and the less weedy, and by the following year three of the July sown plots failed completely. In July 1927 the crop was weighed green and the following yields were obtained:

Treatment	Yield in cwts. per acre	Percentage nitrogen in the crop
Spring sown, inoculated	109·96	2·64
Spring sown, untreated	81·16	2·72
July sown, inoculated	64·2	2·39
July sown, untreated	28·8	2·50

In the trial carried out by Mr C. H. Roberts at Boothby, Brampton, Cumberland, the whole of the July sown plots as well as all the plots sown with untreated seed failed completely. Only the April sown inoculated plots bore any crop, and these produced a fair growth. The experiments agree, therefore, in showing better results from sowing the lucerne in spring with a cover crop than from July sowing. It is probable that in the west at any rate the plant needs a full summer's growth to enable it to meet the competition of autumn weeds. The comparative effects of spring and autumn sowing are no doubt greatly dependent on the weather. Trials sown in different years are therefore still required before the general superiority of the April sown crop can be established.

SUMMARY AND ABSTRACT.

1. The paper discusses experiments laid down at 39 centres in Great Britain to test the value of seed inoculation for lucerne.

2. The seed was inoculated by treating it with a suspension of the nodule bacteria in skim milk containing 0·1 per cent. calcium di-acid phosphate, the method developed by Thornton and Gangulee.

3. In the west and north of England the treatment greatly benefited the lucerne and often enabled a crop to be obtained where the untreated lucerne failed. At 12 centres in this area at which the crop was weighed, inoculation increased the yield by over 20 per cent. in all cases save one, where spread of the bacteria vitiated the result.

4. The improvement sometimes showed itself as an increased yield and sometimes as an increase in the nitrogen content of the hay. In most cases both these effects were produced.

5. In the midland and south central counties inoculation usually produced a temporary improvement, the untreated plant eventually catching up with the inoculated. The effect of inoculation is very much greater where the young lucerne has to compete with a cover crop. Weight results from 8 centres in this area showed increases from inoculation of over 20 per cent. in 4 cases, smaller but significant improve-

ment in yield or nitrogen content in 3 cases, and no significant effect in one case.

6. In East Anglia and Kent untreated lucerne usually develops plenty of nodules. An exceptional condition occurred in a trial at Tunstall Heath, Suffolk, on sour light land, where liming and inoculation produced a fair plant although the uninoculated lucerne developed no nodules and failed.

7. There is evidence that, when the seed is inoculated, the chances of success with lucerne are on the whole as good in the west and north of England as they are in the south-east.

8. In a number of trials sown in 1926 better results were obtained by sowing the seed in a light cover crop in spring than by sowing in June or July.

Acknowledgment.

The author wishes to thank the Royal Agricultural Society of Great Britain for the generous grants of money that have enabled the experiments above discussed to be carried out, the experimenters mentioned, besides others who kindly undertook trials in which the crop failed, and also the Agricultural officers and their assistants who supervised some of the trials and in certain cases carried out the weighing of the crops.

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ON THE INFLUENCE OF THE CARBON : NITROGEN RATIOS OF ORGANIC MATERIAL ON THE MINERALISATION OF NITROGEN.

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(With Four Text-figures.)

Introduction.

The importance of the C : N ratio in biochemical soil processes has received considerable attention in recent years. Doryland (5) first stated definitely that the ammonification by *Bac. mycoides* and other saprophytic soil bacteria is governed by the proportion between the supplies of energy material and nitrogenous food, so that "beneficial" bacteria may become "detrimental" and *vice versa*. Hutchinson and Richards (8) considered especially the stabilisation of nitrogen in organic form during the fermentation of plant materials for the production of "artificial farmyard manure," and found a certain "nitrogen factor," expressing the quantity of N per unit of organic matter utilised by the microorganisms which carry out the fermentation, and generally amounting to 2 per cent. of the dry organic matter. Waksman, on the other hand, considers especially the relationships in the soil and concludes theoretically (10) that organic materials with a N content of 2·0–2·5 per cent. will be decomposed with an immediate production of ammonia, whereas materials with less N will show a shorter or longer lag period or even nitrogen starvation, because all the N here is consumed by the microorganisms which decompose the organic matter. Later on Waksman showed in collaboration with Heukelekian (11) that cellulose-decomposing fungi consume nitrogen in a definite ratio to the amount of cellulose decomposed, namely, one unit of N to every 30–33 units of cellulose. Anderson (1) also found that the most favourable N concentration for cellulose decomposition was 1 unit of N to every 35 units of cellulose, further supply of N being without effect. In quite recent work Waksman

[1] These experiments were started at the State Laboratory of Plant Culture, Lyngby, Denmark, where the soils were kept, and finished at Rothamsted Experimental Station during the writer's stay as a Fellow of the International Education Board. The writer is indebted to Mr H. F. Larsen for sampling the soils and forwarding the samples.

and Tenney (14) found plant materials to be decomposed in soil or sand without any extra consumption of N, if the materials contained at least 1·7 per cent. N. Little attention has been paid to these facts in relation to that important soil process, the mineralisation of farmyard manure, although it has long been well known that addition of straw to the soil tends to check nitrification and causes a more or less temporary decrease in fertility. A noteworthy series of papers on this subject has been published by Barthel and Bengtsson (2), who in their last contribution found that when farmyard manure was added to three different soils no nitrification of the organic N took place in 14 months, whereas the ammonium-N was rapidly and completely nitrified. They sought to explain this by assuming that practically all the organic N was present in the form of bacterial bodies and epithelial cells—compounds which were not easily decomposed. In the present work farmyard manure was compared with a series of other organic materials of varying C : N ratio in order to see whether there exists in farmyard manure the same limiting C : N ratio as in other materials, and whether this limit is different in soils of different reaction, as might be expected, since it is generally assumed that fungi are, at least relatively, more active in acid soil, and since it is well known that fungi, when consuming a given quantity of carbon food, build up more protoplasm and consequently lock up more N than do the bacteria.

Experimental.

The materials used for the decomposition studies were the following: wheat straw (mature); sweet clover (*Melilotus alba*); blue lupin (*Lupinus perennis*); both these plants were cut at the time of full flowering; farmyard manure (pure, fresh cow dung, containing only a trace of ammonia); pea pods (green, of *Pisum sativum*); lucerne (*Medicago sativa,* young plants cut before flowering); and fungus mycelium (fruiting bodies of *Polyporus* sp. (*giganteus*?)). The contents of total carbon (determined by combustion according to Dennstedt (4)) and total nitrogen are given below.

	In finely ground, dry material		
Substance	% N	% C	C : N ratio
Wheat straw	0·54	about 45·0	(84 : 1)
Sweet clover	1·74	45·0	25·9 : 1
Blue lupin	2·26	45·2	20·0 : 1
Farmyard manure	2·33	sample lost at time of C determinations	
Pea pods	2·90	38·4	13·3 : 1
Lucerne	3·46	44·6	12·9 : 1
Fungus mycelium	4·45	45·5	10·2 : 1

Two soils were used for the experiment. One was a light sandy soil,

of strongly acid reaction and poor in organic matter, from an unlimed plot in a liming experiment at the Tylstrup Experimental Station, North Jutland; as little of the soil was available it was diluted with about 30 per cent. of pure quartz sand. This soil had been stored for a long time in an air-dry condition in the laboratory; to ensure the presence of living nitrifying bacteria it was inoculated, at the start of the experiment, with 0·2 per cent. of an acid, sandy forest soil in which nitrification was known to take place. The other soil was a light loamy garden soil of good fertility and faintly alkaline reaction. The soils were used in 600 gm. portions to which 12·0 gm. of the dry organic materials to be tested were added. The initial moisture content of the sandy soil was 12 per cent. and that of the loamy soil 20 per cent.; all the soil portions with additions of organic materials received further 2·0 per cent. water in order to saturate the increased water holding capacity to approximately the same degree as in the control soils. The soil portions were placed in 1500 c.c. Erlenmeyer flasks provided with tight fitting rubber stoppers through which passed a glass tube loosely filled with glass-wool. The flasks were incubated at 25° C. and for the first 15 days kept with the neck downwards in order to facilitate the escape of the CO_2 produced abundantly during the first stages of the decomposition. Samples of about 60 gm. of soil were then drawn at regular intervals, and determinations of NO_3, NH_4 and reaction were made; while at the end of the experiment the humus was determined and estimations were made of nitrogen and methoxyl contained therein. Nitrate was determined by the ordinary Devarda method, ammonia by Bengtsson's (3) KCl method; humus was determined by the method devised by Waksman (12), methoxyl by the ordinary Zeisel method. Reaction determinations were made at the start and for the first two periods by means of the quinhydrone electrode, and since then colorimetrically according to Gillespie. Only the initial and the final *p*H values are given here, the rest being of no special interest.

The results of the nitrate and ammonia determinations are given in Table I, and Figs. 1 and 2 show the amounts of inorganic N, as sum-totals of NO_3N and NH_4N, in acid and alkaline soil, respectively. The acid control soil is seen to show an excellent nitrification, in spite of its strongly acid reaction, but sweet clover and farmyard manure have both *depressed* the amounts of soluble N during the entire period. The pea meal shows some mineralisation of nitrogen, although of the 58 mgm. of N originally added, 42 mgm. remain as organic N after 6 months; and yet of the 2·9 per cent. N in the pea meal only 1·1 per cent., corresponding

to 22 mgm. per 100 gm. of soil, is insoluble in hot water. The fungus mycelium shows an interesting effect: a considerable part of its N is ammonified during the first 2 months of the experiment, but is apparently consumed again during a later decomposition of some non-nitrogenous constituents of the mycelium, so that after 6 months the content of inorganic N is almost the same as in the control soil. In the alkaline

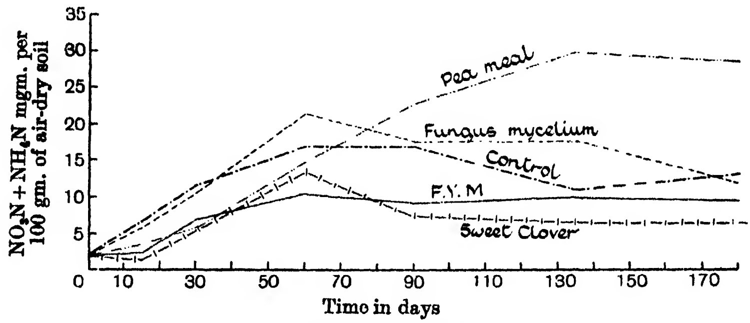

Fig. 1. Mineralisation of nitrogen in acid soil.

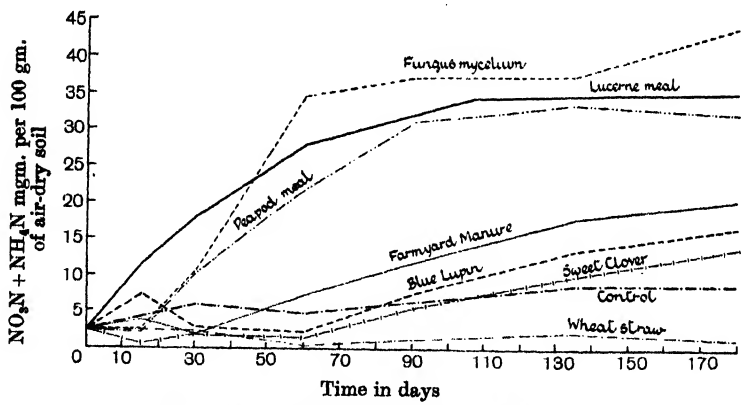

Fig. 2. Mineralisation of nitrogen in alkaline soil.

soil the results are more regular. The wheat straw with its very high C : N ratio gives rise to a constant depression of nitrate and ammonium content, although there is always a little mineral N present, a result supporting Anderson's observation (1), that nitrification and cellulose decomposition may occur simultaneously. The sweet clover has caused a smaller depression during the first 4 months; after 135 days this depression is overcome, and a definite increase in NO_3 content is noted after 6 months. The lupin meal and the farmyard manure show rather

Table I.

I. *Production of NH_4 and NO_3 in acid sandy soil.*

*p*H of soil at start of experiment: 4·74.

Time	1. Control		2. Sweet clover		3. Farmyard manure	
	NO_3N	NH_4N	NO_3N	NH_4N	NO_3N	NH_4N
Start	0·9	1·1	—	—	—	—
After 15 days	—	—	0·0	1·3	0·0	2·4
,, 30 ,,	10·9	0·7	4·9	0·4	5·9	1·4
,, 60 ,,	14·8	2·0	10·5	2·9	9·3	1·5
,, 90 ,,	16·5	0·3	7·4	0·0	9·1	0·0
,, 135 ,,	10·7	0·2	6·5	0·0	9·9	0·0
,, 180 ,,	12·4	0·4	6·4	0·0	8·8	0·6
Final *p*H	4·6		4·8		5·1	

Time	4. Pea pod meal		5. Fungus mycelium	
	NO_3N	NH_4N	NO_3N	NH_4N
Start	—	—	—	—
After 15 days	0·0	3·4	0·0	5·9
,, 30 ,,	2·8	2·9	0·0	10·6
,, 60 ,,	4·1	10·8	4·4	17·1
,, 90 ,,	7·5	15·1	6·3	11·3
,, 135 ,,	21·4	8·2	11·4	6·2
,, 180 ,,	19·0	9·1	9·2	2·6
Final *p*H	4·5		4·9	

II. *Production of NH_4 and NO_3 in alkaline loamy soil.*

*p*H of soil at start of experiment: 7·42.

Time	1. Control		2. Wheat straw		3. Sweet clover		4. Blue lupin	
	NO_3N	NH_4N	NO_3N	NH_4N	NO_3N	NH_4N	NO_3N	NH_4N
Start	1·6	0·7	—	—	—	—	—	—
15 days	—	—	—	—	0·0	0·5	6·9	0·4
30 ,,	6·0	0·0	1·0	1·4	0·8	1·1	2·1	0·7
60 ,,	4·5	0·4	0·0	0·3	1·2	0·2	2·3	0·0
90 ,,	6·4	0·1	1·1	0·0	5·7	0·0	7·7	0·0
135 ,,	9·0	0·0	2·3	0·0	10·0	0·0	13·6	0·0
180 ,,	9·0	0·0	1·4	0·0	13·9	0·0	16·8	0·3
Final *p*H	6·8		7·0		6·8		6·9	

Time	5. Farmyard manure		6. Pea pod meal		7. Lucerne meal		8. Fungus mycelium	
	NO_3N	NH_4N	NO_3N	NH_4N	NO_3N	NH_4N	NO_3N	NH_4N
Start	—	—	—	—	—	—	—	—
15 days	3·5	0·4	0·0	2·2	0·0	11·3	0·0	2·5
30 ,,	0·8	1·0	8·6	1·5	7·4	10·4	0·3	9·9
60 ,,	7·0	0·3	21·7	0·7	27·6	0·2	34·1	0·4
90 ,,	11·9	0·0	31·1	0·1	—	—	37·0	0·2
105 ,,	—	—	—	—	34·4	0·0	—	—
135 ,,	17·9	0·0	33·6	0·0	—	—	37·3	0·1
150 ,,	—	—	—	—	35·2	0·0	—	—
180 ,,	20·6	0·3	32·3	0·2	35·2	0·0	44·3	0·0
Final *p*H	6·9		7·0		6·7		6·9	

more nitrification, the latter more than the former; unfortunately the carbon content of the manure could not be determined, but if a content of 42 per cent. is assumed as not unreasonable, we arrive at a C : N ratio of about 18 : 1. When we pass from this value to that of the pea meal, viz. 13·3, a large increase in the nitrate production is observed. The lucerne meal, in which the C : N ratio is only slightly lower than

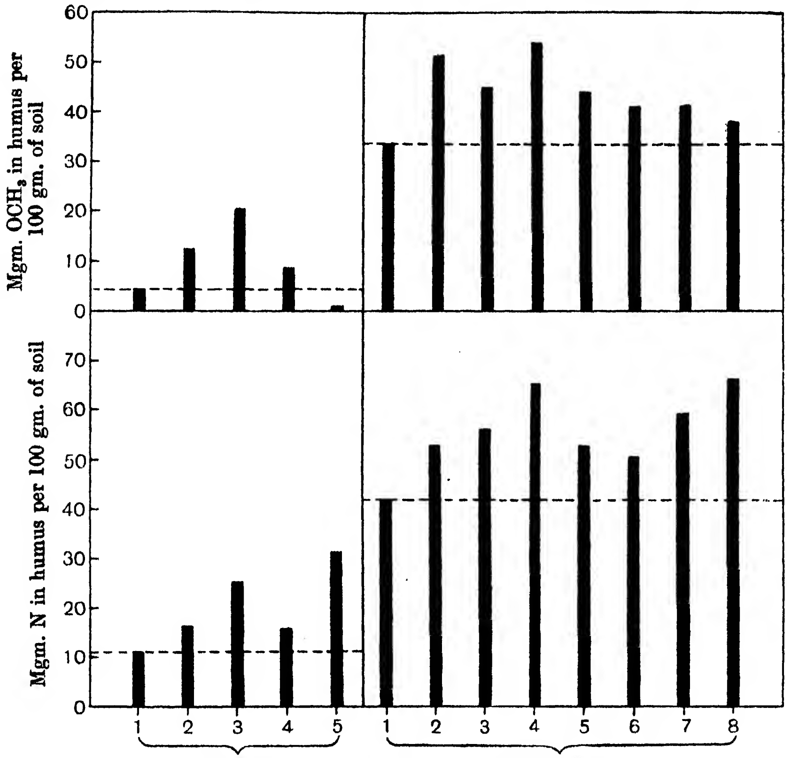

Fig. 3. Amounts of nitrogen and methoxyl in humus.

that of the pea meal, has shown a nitrification which is only slightly stronger, in spite of its considerably higher N content. Finally, the fungus mycelium with the most narrow C : N ratio behaves in an interesting manner: after a lag period there follows a very rapid production of mineral N which after 3 months comes almost to a standstill. There is thus a correlation between the C : N ratios and mineralisation of the nitrogen, the ratio of about 26 : 1 being about the limit above which no nitrification will begin within a period of 6 months in alkaline soil

under the conditions of the experiment; in the acid soil the limit lies much higher—somewhere between 18·1 and 13·3 : 1. It should be noted, however, that the time is a factor of considerable importance. In Fig. 3 the amounts of inorganic N in the alkaline soil after 1, 2, and 6 months are plotted against the contents of N in percentage of C. After 1 month there is a sharp break of the curve between the figures for pea and lucerne meal, but after 2 months the break is most pronounced between lupin and pea, and the former break is less marked. After 6 months the lag period for sweet clover has been overcome, and the two other breaks have almost disappeared. The reason why the fungus mycelium falls out of the range of the other substances is probably the presence of an undecomposable nitrogenous fraction, as mentioned below.

Further it is of interest to note that in the case of substances rich in N one part of the N is mineralised rapidly, another only very slowly, the NO_3 figures becoming practically constant for pea and lucerne meal after 3–3½ months; the same holds true of fungus mycelium, although this curve is a little more irregular. If the increase in NO_3 content over control is taken as an index of the amount of NO_3 produced from the organic matter, it is possible to calculate the amount of unnitrified N by substracting this excess from the total amount of N in the organic matter; this calculation shows that the residues of N after 6 months are fairly constant in comparison with the amounts originally added and correspond to 1·5–2·2 per cent. of the original material, increasing with decreasing C : N ratio:

Substance	N added mgm.	N un-nitrified	% N original	% N Nitrified	% N Unnitrified
Sweet clover	34·8	29·9	1·74	0·24	1·50
Blue lupin	45·2	37·1	2·26	0·40	1·86
Manure	46·6	35·0	2·33	0·55	1·75
Pea pods	58·0	34·5	2·90	1·17	1·75
Lucerne	69·2	43·0	3·46	1·37	2·15
Fungus mycelium	89·0	44·7	4·45	2·21	2·24

To determine whether a part of this not readily nitrified N has passed into the soil humus the amounts of "α-humus" (crude mixture of humic and hymatomelanic acid) were determined according to Waksman (12); the determinations were carried out in triplicates, and 2 portions of humus were used for N determinations, 1 for methoxyl determination. Table II gives the results.

The acid soil contains only a little "α-humus" which is remarkably poor in N and methoxyl; sweet clover gives a slight and farmyard manure a very marked increase in humus, and both materials, especially

the latter, increase the percentage of both N and methoxyl; pea pods have not given any significant increase in the actual amount of humus, but the percentages of N and methoxyl are also here considerably increased; the fungus mycelium has increased the humus content almost as much as the farmyard manure, but what is especially noteworthy is the extraordinary increase in N content and the reduction of the methoxyl content. The garden soil is much richer in humus which also contains more N and methoxyl. The wheat straw has given a considerable increase in amount of humus, a slight decrease in N content and a very distinct

Table II. *Amounts and composition of humus in soils.*

	Humus % in air-dry soil	N % in humus	OCH_3 % in humus
I. Acid soil:			
1. Control	0·49	2·43	0·86
2. Sweet clover	0·57	2·86	2·13
3. Farmyard manure	0·83	3·03	2·43
4. Pea pods	0·51	3·07	1·68
5. Fungus mycelium	0·72	4·37	0·11
II. Alkaline soil:			
1. Control	1·05	4·00	3·20
2. Wheat straw	1·36	3·89	3·73
3. Sweet clover	1·32	4·24	3·40
4. Blue lupin	1·63	4·00	3·27
5. Farmyard manure	1·36	3·85	3·22
6. Pea pods	1·24	4·08	3·31
7. Lucerne	1·29	4·58	3·19
8. Fungus mycelium	1·33	4·97	2·86

increase in methoxyl content—a fact which would indeed be expected from a substance rich in lignin. The sweet clover has here given a much greater increase in humus than in acid soil; the N content, too, is increased considerably, the methoxyl only a little. The blue lupin has given a very large increase in humus, with no significant changes in N and methoxyl percentages (although the actual *amounts* of N and methoxyl in humus are increased). The manure has given almost the same increase in humus and reduction of N content as the straw, but has not affected the methoxyl content. The pea pod meal, deficient in lignin, has given only a small increase in humus, with almost unaltered N and methoxyl percentages. The lucerne meal, also poor in lignin, has increased the humus only a little and left the methoxyl content unchanged, but has given a very marked increase in N content. Finally the fungus mycelium has given a moderate increase in humus, as in the acid soil a striking increase in N content and a decrease in methoxyl. The original mycelium contains a fraction, amounting to about 12 per

cent. of air-dry matter, which shows great similarity to the "humic acid" or α-humus of the soil: an amorphous, dark brown substance, soluble in alkalies with intensely black colour and precipitated by acids as a voluminous, flocculent gel; in dry condition it contains 5·08 per cent. N, 55·4 per cent. C, and only a trace of methoxyl. Like the soil humus it is very resistant to the attack of soil microorganisms, so that its N is not readily nitrified in spite of its narrow C : N ratio (11 : 1); in an experiment—to be described in a later contribution—where 1 per cent. of the substance was added to a fertile, faintly alkaline garden soil, no increased nitrification over control occurred within 5 months.

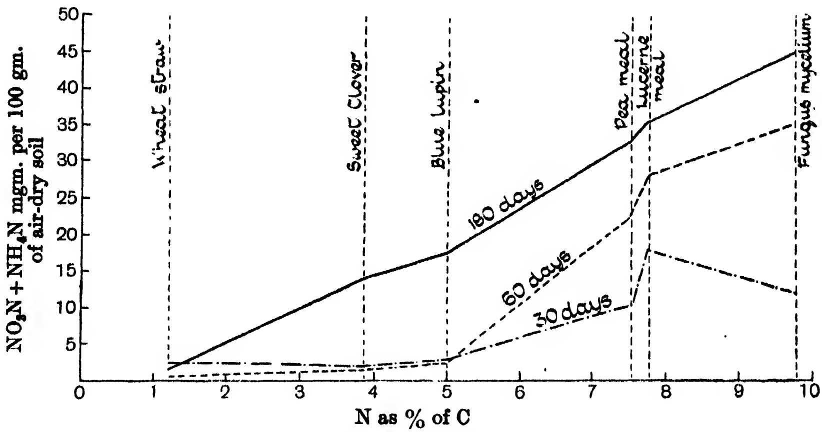

Fig. 4. Mineralisation of nitrogen in relation to composition of organic materials.

The amounts of nitrogen and methoxyl in humus in the differently treated soils are shown in Fig. 3. It seems that all the materials except the fungus mycelium have increased the humus content partly through their lignin content, partly in another manner which accounts for the increases in humic nitrogen. That lignin is a mother substance of soil humus can now, according to the studies of Fischer (7), Waksman (12), and du Toit (6), be regarded as settled beyond doubt; the origin of the nitrogen in the humus is somewhat more obscure; Waksman (12, 13) seeks the explanation for this in the synthesising actions of microorganisms, especially fungi which decompose cellulose actively in the soil and build up large amounts of protoplasm, and has actually shown (13) that a nitrogenous humus-like substance arises out of pure cellulose decomposing in pure sand with addition of mineral nutrients; the present results seem to lend some support to this theory when one considers the

increase in humus-N following the decomposition of such cellulose-rich substances as sweet clover and blue lupin, and lignin-poor substances as pea pod meal and young lucerne plants. Waksman (12) considers this nitrogenous humus a normal constituent of microbial protoplasm in general and fungus mycelium in particular; the present experiments furnish the evidence that *Polyporus* does indeed contain such a "humus"-fraction. But whether the formation of such substances is a general property of microorganisms can hardly yet be regarded as settled.

Conclusions.

The most striking result of the experiments is the fact that in general the C : N ratio of the added material influences the nitrification as much as does the soil reaction, and that in alkaline soil a certain part of the nitrogen added to the soil, namely one part of N for each 20–25 parts of C added along with the N, is nitrified only very slowly, whereas the excess over this is liberated quite readily. In acid soil the limit is much higher, viz. one part of N for each 13–18 parts of C, probably on account of the greater activity of the fungi which synthesise more protoplasm and consequently store up more nitrogen than the bacteria. The results thus conform well with those of Waksman and Tenney (14) and have a very interesting bearing upon the utilisation of the nitrogen in farmyard manure; the very slow nitrification of the last 1·5–2·0 per cent. of nitrogen is apparently the cause of the smaller effect of farmyard manure nitrogen in comparison with N as nitrate of soda, and the very rapidly decreasing nitrifiability which follows a decrease in N content towards 2 per cent. of the organic matter conforms well with the observation of Iversen (9), that a loss of about 25 per cent. of the total N of farmyard manure implies a reduction of about 50 per cent. of the fertilising value of the manure in the first year; thus the nitrogen which is present in excess over what corresponds to a C : N ratio of 20 : 1 is the most valuable part and should be most carefully guarded against loss. Barthel and Bengtsson (2) found no nitrification of the organic N of farmyard manure (amounting to 2·12 per cent. of dry matter), but in this case the nitrification of the organic N of manure did occur, only 1·75 of the original 2·33 per cent. N being left unnitrified after 6 months. It should be remembered, however, that *fresh* manure was used, whereas Barthel and Bengtsson worked with old, well-rotted manure in which highly resistant nitrogenous compounds ("humus") may possibly have been formed during the fermentation.

Finally it is interesting to note that in the case of manure more of

the N has been found in the "humus" in the acid than in the alkaline soil, and in the former soil it should be still less available to the plants, because the humus of the acid soil has a lower N content than that of the alkaline soil and consequently is presumably still more slowly nitrified.

Summary.

Organic materials with a C : N ratio ranging from about 85 : 1 to about 10 : 1 were submitted to nitrification tests in an acid and in an alkaline soil during a period of 6 months. In the acid soil only pea pod meal, with a C : N ratio of 13·3 : 1 showed an increase in inorganic N over control; in the alkaline soil the limit above which no nitrification will occur within a period of 6 months was at C : N = 26 : 1; below this limit the rate of nitrification increased rapidly with decreasing C : N ratio. Unnitrified N was left behind in a quantity corresponding to 1·5–2·2 per cent. of the original material, the percentage being higher in the case of materials rich in N.

All the materials tended to increase the content of "α-humus" in the soil, though not to the same extent or in the same manner. More "α-humus" was produced in the alkaline than in the acid soil, except in the case of farmyard manure. Straw, sweet clover, lupin and farmyard manure apparently acted both through their lignin content and through the synthesising action of microorganisms, since they increased the amounts of both N and methoxyl in humus. Mycelium of *Polyporus* contains a fraction possessing the properties of "humic acid," rich in N, but devoid of methoxyl, which persists in the soil.

As a general result the experiments show that the carbon : nitrogen ratio is a factor which exerts an influence on nitrification as profound as that of soil reaction, and that the less complete utilisation of farmyard manure nitrogen as compared with nitrogen in artificial fertilisers can to a large extent be explained hereby.

The writer's best thanks are due to Sir John Russell, D.Sc., F.R.S., for placing the facilities of the laboratories at his disposal; to Mr H. G. Thornton, Head of the Bacteriology Department, for discussing the experimental data and reading the manuscript; to Dr R. A. Fisher, Head of the Statistical Department, for advice and assistance; and to Miss S. Heinze, mag. fil., and Mr G. V. Jacks, B.A., B.Sc., for assistance in carrying out the carbon and methoxyl determinations.

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LIMING AS A FACTOR IN THE AMELIORATION OF DETERIORATED TROPICAL SOILS.

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I. Introduction.

Tropical soils, when brought into cultivation, frequently undergo rapid deterioration, due, as a rule, to one or more of the following causes: (*a*) neglect of crop rotation, or the practice of a primitive inefficient rotation by abandoning the land to bush between successive plantings, (*b*) use of imperfect methods of tillage and drainage, and (*c*) failure to replace essential soil constituents removed by erosion and leaching, and by the crop. The exhaustion of the soil, which results from such neglect of sound agricultural procedure, is characterised by the destruction of its tilth, the appearance of disease, and a diminution in crop yields.

Within the cane-growing regions of Trinidad there exist large areas of worn-out soils, on which the sugar-cane is subject to serious injury from a complex of diseases known as "froghopper blight." From these diseases the canes on soils in good condition are comparatively free. Of recent years the damage from blighting has been sufficiently severe to warrant an investigation of the factors which determine the condition of the deteriorated soils, to ascertain the measures most suitable for their amelioration.

A comparison of the exhausted soils with those which still retain their fertility has demonstrated that the dissimilarity in their properties can rarely be explained by the difference in their humus content. The former contain from 1·8 to 6·0 per cent. of organic matter, the latter from 2·1 to 6·8 per cent. (7). Both, as a rule, are poor in humus, and both respond to application of pen manure.

The examination, however, has shown that the two soil groups differ greatly in their lime status. The soil types which have retained their fertility, even after long periods of continuous cultivation, are either naturally calcareous, and still possess a reserve of calcium carbonate, or they have been so liberally limed in past years that they are now in equilibrium with this substance. The deteriorated soil types are devoid of calcium carbonate. They are, in general, markedly acidic (2). By comparison with fertile soils of similar type and texture they are

definitely deficient in exchangeable calcium, notwithstanding the fact that they frequently contain as much as 0·30 gm. (calculated as oxide) per 100 gm. of air-dry soil (7). So marked is the difference in this respect that a material improvement in the lime status of the worn-out soils appears to be a necessary preliminary to the restoration of their fertility.

In view of this, an investigation has been made of the methods by which the content of exchangeable calcium of the deteriorated soils may most efficiently be increased. Advantage has been taken of the existence in the island of a carefully planned series of experimental liming plots, set up in 1923 by G. A. Jones, with the object of determining the relative values of finely ground limestone and slaked lime as soil ameliorants (4).

II. The Experimental Plots.

The site chosen for the experiment is an area of an alluvial soil type which has been under sugar-cane cultivation for generations. The soil is heavy and compact in texture, and is frequently mottled just below the surface. It exhibits marked fluctuations in behaviour from season to season. In dry weather it sets like concrete and cracks badly. If the weather remains fine after ploughing the top few inches of soil alone cake. The sub-surface soil remains loose, but its individual aggregate particles set as hard as stone. On the advent of rain the soil assumes the consistency of putty, and water oozes from it under the pressure of the foot.

Some physical constants of this soil are recorded in Table I. There are also included in this table the constants of a composite soil sample from an area within the same soil type, the soil of which exhibits much less pronounced changes in behaviour in the dry and wet seasons, and retains, by comparison, a good tilth throughout the year.

Table I. *Physical constants of deteriorated and fertile soils.*

	Deteriorated soil (Plot 1)	Fertile soil
Specific gravity	2·41	2·48
Water capacity	0·86	0·85
Pore space	66·9	66·0
Volume expansion	14·17	25·10
Moisture content (air-dry soil)	5·61	6·18
Hygroscopic coefficient	8·84	8·86
Moisture at point of stickiness	41·1	37·8
Dispersion factor	10·5	9·1
Organic matter	3·8	4·6

Each of the values presented in Table I is the mean of a number of closely agreeing determinations. The corresponding constants of the two

contrasted soils differ greatly in one respect only. The volume expansion of unit volume (100 c.c.) of the deteriorated soil, when saturated with water, is much smaller than that of the fertile soil. The values of this constant alone reflect the magnitude of the difference in the field behaviour of the two soils. It is noteworthy that Stead (5), in his examination of new and worn-out pineapple soils, found no marked difference in their pore space or water retentivity.

The mechanical composition of the soil from the experimental plots is given in Table II. The soil is remarkable in that it is devoid of stones and gravel, and in that sand is present in very small quantities only. Its content of clay and fine silt is sufficiently large to place it in a very heavy class.

Table II. *Mechanical composition of the plot soils.*

	Moisture	Loss on ignition	Coarse sand	Fine sand	Silt	Fine silt I	Fine silt II	Clay	Total
Top 6 in.	5·44	7·94	1·48	4·20	12·83	16·07	11·53	40·42	99·91
Second 6 in.	5·86	7·44	1·99	3·50	10·50	19·69	12·31	38·92	100·21
Second foot	6·02	6·76	0·60	2·55	9·80	16·75	13·00	44·75	100·23

The soil of the experimental area appears to be very uniform in character. The saturation capacity for calcium-ions, as determined by the modified method of Page and Williams (6), of the top 6 in. of soil from the various plots varies only from 0·59 to 0·62 gm. of calcium oxide per 100 gm. of air-dry soil. The mean saturation capacities of the second 6 in. and second foot of soil are 0·62 and 0·52 gm. respectively.

The experimental plots are each one-thirtieth of an acre in extent. The treatments to which they have been subjected are detailed in Table III. Nine replicates of each plot receiving a particular treatment exist. The plot scatter is not ideal, but the uniformity of the soil renders this unimportant. The determinations of the plot soil contents of exchangeable calcium were made in 1927, over three years after the initiation of the experiments. The analyses were performed, by the standard method of Hissink (3), on composite samples of 18 borings from each series of replicate plots.

Table III. *Effect of liming on soil content of exchangeable calcium.*

Treatment per acre	Content of exchangeable calcium (gm. CaO per 100 gm. air-dry soil)		
	Top 6 in.	Second 6 in.	Second foot
1. Control (unlimed)	0·32	0·30	0·23
7. 10 cwt. lime in 1924, 1925, 1926	0·35	0·31	0·22
8. 1 ton limestone in 1924, 1925, 1926	0·42	0·29	—
9. 4 tons lime in 1924 only	0·36	0·28	—
10. 8 tons limestone in 1924 only	0·49	0·35	0·25

III. Experimental Results.

The data in Table III indicate that:

(*a*) Finely ground limestone reacts more rapidly than slaked lime with the deteriorated soil, whether the applications are large or small. This is contrary to experience in England, where equivalent quantities of limestone and lime are known to differ but little in efficiency.

(*b*) Both with limestone and slaked lime, large single applications give more promising results than small annual treatments. Within four years the degree of saturation with calcium-ions, of the top 6 in. of soil of plot 10, has been raised from 54 to 80 per cent. It is noteworthy that the top foot of the fertile soils of the island has invariably been found to be approximately 80 per cent. saturated with calcium-ions.

(*c*) The effect of liming has penetrated below the top 6 in. of soil only on those plots which have received an application of 8 tons of ground limestone per acre. Even on them the increase in the content of exchangeable calcium of the second 6 in. is very small, whilst that of the second foot is negligible. The absence of appreciable reaction with the sub-surface soil is doubtless partly due to the impermeability and lack of aeration which characterises deteriorated soils of heavy texture. So compact are these soils that the effect of liming, in the majority of cases, has extended merely to the depth to which they are worked.

Very large areas of alluvial soils are under cultivation in the tropics. It is possible that the experience of liming methods gained in Trinidad may be applicable to them.

IV. Effect of Liming on Soil Acidity.

The *p*H values[1] of the plot soils, which were determined with the quinhydrone electrode, are recorded in Table IV. The data indicate that the decrease in acidity of the soils of the various plots is closely related to the increase in their content of exchangeable calcium.

Table IV. *Effect of liming on soil reaction.*

	*p*H values		
Treatment	Top 6 in.	Second 6 in.	Second foot
1	5·72	5·55	5·18
7	6·00	5·82	5·13
8	6·65	5·48	5·12
9	6·40	5·52	5·03
10	7·02	5·45	5·07

[1] The author is indebted to Mr Follett-Smith for these determinations.

V. EFFECT OF LIMING ON THE YIELD OF SUGAR-CANE.

The yields of the sugar-cane on the experimental plots for the years 1925 and 1926 are presented in Table V. The corresponding data for 1927 are not available.

Table V. *Effect of liming on yields of cane, expressed as quarters per plot.* (G. A. Jones.)

(1) *Yields of Plant Canes* (1925).

Treatment	Plot (a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	Mean
1	110	108	112	93	102	98	108	94	102	103
7	122	97	114	103	88	90	101	114	117	105
8	122	119	116	101	95	119	106	89	96	107
9	116	115	119	115	103	94	113	89	108	108
10	113	106	108	116	97	121	112	122	102	111
Mean	117	109	114	106	97	104	108	102	105	107

(2) *Yield of Ratoon Canes* (1926).

Treatment	Plot (a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	Mean
1	43	44	49	51	40	43	48	43	47	45
7	54	45	55	56	40	39	45	47	44	47
8	31	51	50	55	43	43	44	57	56	48
9	41	43	51	46	45	48	59	52	54	49
10	51	61	55	54	45	50	47	43	62	52
Mean	44	49	52	52	43	45	49	48	53	48

The treatments 1 and 10 give the lowest and highest yields, respectively, of both plant and ratoon canes. Comparison of the yields of cane in 1925, on the most closely proximate plots of each of these treatments, demonstrates that their mean difference of 8 qr. is almost twice its standard error[1], 4·1 qr. P is therefore approximately equal to 0·05 and the difference of 8 qr. may be taken as significant. For the yield of canes in 1926 the corresponding mean difference is 7 qr., and its standard error is 2·6 qr. The difference in this case is certainly significant, P being less than 0·01.

None of the treatments, other than 10, give a significant increase in yield over the control. In view of this, it is worthy of note that treat-

[1] The statistical examination was performed according to the method suggested by Engledow and Yule (1). The standard error of the mean difference, $\frac{\sigma_d}{\sqrt{n}}$, was determined from the equation $\sigma_d^2 = \frac{2m}{m-1}(\sigma_v^2 - \sigma_p^2)$, where n = number of plots of the same treatment, m = number of treatments, σ_v = standard deviation of the yield for a treatment from its own mean, and σ_p = standard deviation of the means for plots of the same number.

ment 10 alone resulted in a sufficient increase in the content of exchangeable calcium of the top 6 in. of soil, by the year 1927, to raise its degree of saturation with lime to a value comparable with that of the fertile soils of the island, and to render it neutral in reaction.

Summary.

1. There exist in Trinidad large areas of deteriorated soils which have been under cultivation with a single short-term crop for generations. It has previously been established that these soils differ but little from those which still retain their fertility in their content of organic matter. By comparison with the latter, however, they are markedly acidic and so deficient in exchangeable calcium that a material improvement in their lime status appears to be a necessary preliminary to their amelioration. An examination has therefore been made of the means by which this may most successfully be accomplished.

2. Determinations of the contents of exchangeable calcium and the pH values of a series of liming experimental plots indicate that:

(*a*) Contrary to experience in England, finely ground limestone has proved a more efficient soil ameliorant than slaked lime.

(*b*) Single relatively large applications of lime fertilisers have given more immediate beneficial results than small annual dressings.

(*c*) The effect of liming appears to have been almost entirely restricted to the depth to which the soil is worked. This is doubtless due to the impermeability and lack of aeration which characterises heavy deteriorated soils.

3. A significant increase in crop yield was obtained only on those plots which were later shown to have been rendered neutral in reaction by liming, and on which the degree of saturation of the top 6 in. of soil has been raised to 80 per cent. This value is comparable with that of the fertile soils of Trinidad.

4. The experience of liming methods gained in Trinidad may be applicable to other areas of alluvial soils under cultivation in the tropics.

Acknowledgments.

The author is indebted to Mr G. A. Jones of the Usine Ste Madeleine Estates, Ltd., for permission to examine his experimental liming plots, and to publish the data recorded in Table V.

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STUDIES IN THE GEOLOGY AND MINERALOGY OF SOILS.

I. A DETAILED STUDY OF A REGION CHARACTERISED BY DIVERSE ROCKS AND PARTLY COVERED BY GLACIAL DRIFT.

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(With One Fig. and Plate I.)

The classification of soils has been attempted in a variety of ways, and although it is now generally recognised that climate probably forms the basis of the broadest classification yet the geological nature of the parent material is very important, especially in a country like Britain, in which the chief problem is the subdivision of the climatic types.

The present work is a detailed investigation of the geology and mineralogy of the soils of a small area, specially selected because of the varied geology of the parent material. The rocks of the district are very diverse and are mainly overlain by a covering of glacial drift, which is itself varied in character. Partly because of this and partly because of the topography the soils are very varied. The area comprises a farm at Boghall, Midlothian, Scotland, owned by the Edinburgh and East of Scotland College of Agriculture and is typical of many Scottish hill-foot farms, consisting of a cultivated area at the base of the hill and a sheep-run stretching to the top.

Hall and Russell (1) in a survey of the soils of Kent, Surrey and Sussex, found that their soil-type boundary lines coincided with the boundaries of geological formations, so that they could say (p. iv): "all our experience in the field goes to show that each (geological) formation in the area under consideration gives rise to a distinct soil-type...characterised by its mechanical analysis." It should be noted that texture is the main point considered. The soils of the above area, however, differ very much in their geological aspect from those in the greater part of Britain. They are mainly residual soils, that is, derived directly from the underlying rocks, whereas the majority of soils in this country are developed from "drift" material, either boulder clay or glacial sands and gravels, and the problem is more complicated in such regions.

The Geological Survey have published Memoirs on certain districts in which short accounts may be had of the soils and explanations of the

Recent and post-Tertiary formations. Maps have also been issued showing these deposits. But generally the information on soils is not detailed, and much more detailed information is needed, not only of the texture of the materials but also of their petrographic nature and their relationship to the underlying rock formations.

The effect of the petrographic nature of the geological formation on the soil was shown by T. Rigg (2) in a survey of an area in which glacial deposits occur, though to a minor extent. He showed that each soil series coincided in area with a geological formation and "that the geological formations give rise to a series of related soils, which exhibit this relationship more closely in the *mineral constituents* than in the texture." More recently, Hendrick and Newlands (3, 4) in a study of the mineralogical composition of certain Scottish soils of glacial drift origin showed that the soils could be graded according to their ferro-magnesian silicate content, and that in certain areas the nature of the drift (boulder clay) is almost invariably determined by the kind of rock which had been immediately traversed by the ice.

Ogg (5) has also pointed out that though a broad classification of Scottish soils is possible on a climatic basis, the geologic nature of the parent material must also be taken into account, but that through lack of information of the nature of the matrix of the glacial drift, correlation cannot yet be carried out.

It would seem that before a classification of the soils of both Scotland and England can be carried out a knowledge of the petrographic character of the matrix of the glacial formations on which they lie is necessary. References to the development of methods of examining soil minerals may be found in the papers by Hendrick and Newlands already cited and also in a paper by H. Loos (6) on the soils of Java and Sumatra, where an extensive bibliography is given.

PHYSIOGRAPHY.

The Experimental Farm, Boghall, Midlothian, lies to the south-west of Edinburgh on the south-east slope of the Pentland Hills. The acreage is about 600, of which 200 are given over to arable land and the remainder to pasture.

The arable land is rolling in appearance and rises from 500 feet at Seafield to 700 feet at the Steading. The mounds trend roughly north-east to south-west.

The pasture land rising from 700 feet to 1615 feet consists of the glen, the bottom of which is fairly flat, and the actual hill slopes of

Allermuir and Caerketton Hills which are steep. From the Steading the land rises gradually to the north-west till about 1250 feet, where the slope to Caerketton Hill then steepens. This is also the case in the glen itself, so that on the south and south-west slopes of Caerketton and on the southern slopes of Allermuir Hill, screes appear at about 1250 feet. The flat bottom of the glen, most extensive at the base of Allermuir Hill, is diversified at the lower end by mounds of gravel banked on the side of the hill and cut by two small streams running into the main burn.

Climate.

A general account of the climate of Midlothian is given by McCallum (7).

The county, by reason of its topography, is divided into two well-marked climatic areas, one a region of rainfall less than 30 in. and the other of rainfall from 30 in. to 40 in. Boghall lies in the second region. Climatic records at the farm only extend from 1925, so that no figures for a long period can be given. The annual rainfall from 1925–8 averaged 38·7 in., while the average annual rainfall at Edinburgh over a long period is only 25·4 in., but over the whole county great variations occur, caused by variations in altitude. The sunshine averaged 3·7 hours per day over the period 1925–8 while the average monthly temperature for the same period was 46·5° F.

Geology.

A general description of the rocks of Midlothian in which the farm is situated has been published by the Geological Survey of Scotland in their Memoir, *The Neighbourhood of Edinburgh* (8). The solid rocks of the farm area belong to the Lower Old Red Sandstone and the Carboniferous formations, and these are partly overlain by a series of glacial sands, gravels and clay.

Formations and Rock Groups.

The geological formations occurring in the area are:

1. Pleistocene and Recent	Peat. Alluvia. Glacial sands and gravels. Boulder clay.
2. Carboniferous	Calciferous Sandstone series.
3. Lower Old Red Sandstone	Lavas. Tuffs.

The rocks of Lower Old Red Sandstone age are most extensive and stretch from Allermuir Hill through Caerketton to a point about 300 yards north-west of Seafield. They are volcanic rocks, being a series of lava flows and tuffs dipping roughly to the south-east. They are of varied character and may be grouped as follows, starting from the youngest:

(*a*) Allermuir group of basic andesites and basalts.

(*b*) Caerketton group of rhyolitic lavas, acid andesites, trachyte and tuff.

(*c*) Caerketton group of basalts and basic andesites.

All these rocks are in a highly decomposed state and many secondary minerals have been developed in them. A detailed account of the rocks is given in the Memoir (8), p. 29.

The Allermuir group of basalts and andesites is dark red in colour, being heavily stained with iron oxides. The olivine and augite have decomposed. Haematite, chlorite and calcite are the secondary minerals developed.

The Caerketton rhyolite has been completely silicified though the original rock structure has been retained. It is composed of fine-grained felsitic material.

The trachyte included in this band contains biotite and sanidine, while zircon, iron oxides and apatite are found in the ground mass.

The third group of lavas resembles the Allermuir group but is more basic in character. Olivine is represented by pseudomorphs and plagioclase felspar is very abundant. Augite and hypersthene andesites also occur. Decomposition products such as iron oxides, chlorite and calcite are present.

The Upper Old Red Sandstone is not represented in this area, and the junction between the lavas and the Carboniferous formation is here a reversed fault, the Pentland Fault, which separates the Pentland Hills from the Midlothian coal-field to the east. On the farm ground the position of the fault can only be inferred, since the ground is obscured by drift deposits. The rocks to the south of the fault consist of sandstones and shales belonging to the Calciferous Sandstone series, that is, the lowest group in the Carboniferous formation.

During the Glacial Period Midlothian was invaded by ice from the north-west, but it was also affected by ice from the south. The ice from the north-west completely covered the Pentland Hills and left as relics a deposit of boulder clay, erratics, sands and gravels.

From the map (Fig. 1) it will be seen that a great part is covered

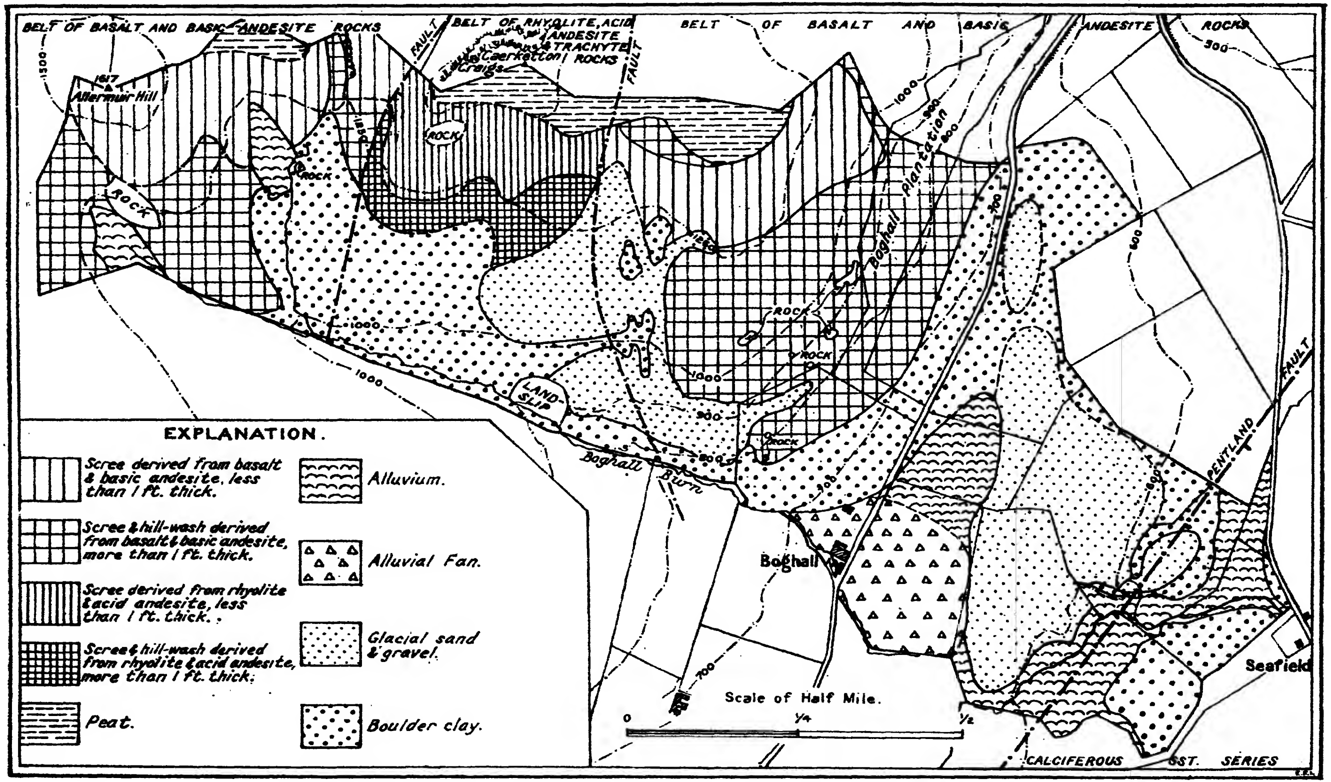

Fig. 1. Map of geology (drift and solid) of Experimental Farm, Boghall, Midlothian.

by these drift deposits, and it is only on the higher ground that the bed-rocks are seen. But even here on the hill tops, where the soil at present is very thin and rests on shattered rock, glacial material may well have been spread at one time and then mostly removed by subsequent denudation, as is indicated by a soil analysis quoted later. Bennie (9) has described the finding of a boulder clay on the top of Allermuir Hill, but this has not been confirmed.

The Boulder Clay.

The boulder clay is the most extensive glacial deposit and covers most of the ground from Seafield to the base of Allermuir, although in part it is covered by later deposits. It is fairly uniform in texture but tends to be sandier on the hill slopes. Two isolated patches of it occur on the side of Caerketton Hill, indicating probably a greater extent at one time.

In the glen good exposures of the clay can be seen and it is there reddish-brown in colour and, for a boulder clay, fairly loose in texture. The included stones are not large in size and consist mainly of fragments of lavas of the Pentland Hills, with a subordinate amount of quartzite, schists, grits, sandstones, and shale. The quartzites and schists are derived probably from the South-west Highlands. At the Seafield end of the farm the clay is greyer in colour and there is a greater amount of sandstone, shale and coaly material, but the Pentland lavas are still prominent. The boulder clay here overlies rocks of the Carboniferous formation.

Sands and Gravels.

Overlying the boulder clay occur two large spreads of sands and gravels, one on the arable land, the other in the glen. The spread to the south of the road forms a series of mounds trending north-east to south-west. It is very variable in character, having patches of gravel with intervening lenticles of sand, and at other parts clay. This is well seen after ploughing, when the furrows stand up well in the clayey parts but not so well in the others. The pebbles in the gravel vary in size from ordinary gravel to stones about 3 in. in diameter. As in the boulder clay the predominant pebbles are local lavas, but sandstones, shales, quartzites schists, grits and coal are also present. There is no well-marked transition here from the gravel to the boulder clay, and the gravelly nature of the ground to the south-east may well be due to weathering of the clay and local segregation of the boulders.

The gravel-spread in the glen overlaps the boulder clay and is itself overlapped by hill-wash from Caerketton Hill and Allermuir Hill. It is much stonier than the lower spread and the predominant pebbles are lavas. In both spreads the stones are markedly rounded in contrast to the angular stones of the scree and the boulder clay.

Post-Glacial Deposits.

Alluvium. The burn which runs down the glen has in parts cut its way down through the boulder clay to expose the bed-rock. Two streams, flowing into the burn from the north-west, have also dissected the boulder clay. In its upper part the gradient of the burn is such that no material is deposited and erosion is still going on. In the field, however, the gradient is very much lessened so that behind the Steading and extending along the banks of the stream to Seafield occurs a spread of alluvium. In the more easterly part the alluvium is of a silty character, but at the Steading it is of the nature of an alluvial fan with much stony material. To the north-west of this fan the ground rises slightly to form a small flat which probably consists of alluvial material laid down by the flooding of the burn. Similar fans may be seen in process of formation at the confluences of the two tributary streams to the main stream in the glen.

At the head of the glen on the slope of Allermuir Hill there are two small patches of alluvium consisting mainly of hill-wash, and in a small hollow on the south slope of Caerketton a similar deposit occurs.

The scree material. As one ascends Caerketton Hill from the road and crosses the belt of boulder clay, the surface of the ground is seen to get stonier, the stones being very angular. These have been derived from material slipping down the hill. Off the boulder clay and forming an extensive belt at the plantation the scree material is mixed with hill-wash and is more than 1 ft. thick. Higher up the hill occurs another belt of scree but much thinner and with less soil. The scree material here, of course, is basaltic and andesitic in character, so that with the reservations already made, the soils are practically residual.

Further up the glen the same features can be observed on the slopes of Caerketton and Allermuir Hills, the steep brow of Caerketton standing out prominently at one point with its white screes. At the base of Allermuir Hill the screes are very thick and have formed a shoulder overlapping the boulder clay.

At various points among the scree, bare rock sticks out, but this

occurs nowhere else on the farm. The soil is thin and in the higher parts the screes are still unstable.

Peat. On the tops of Allermuir Hill and Caerketton Hill peat occurs, but it is very thin and at no part thicker than about 6 in. There is generally very little mineral soil on the flat tops, but it is present on the basaltic slopes under the peat. The screes of Caerketton Hill have a peaty matrix. Patches of peaty soil also occur in the hollows between the mounds on the arable ground.

Description of Soils.

In Table I is given a list of the soils examined and their localities, with a note on the underlying rock formations. It will be noted that with the exception of one soil (Sample I), all the soils are derived from drift material. Chemical data on certain of these soils are given by Smith (10) and by Ogg and Dow (11).

Table I. *List of soils examined.*

Soil	Locality	Description of surface soil	Geology
A.	Croft's Field	Brown and chocolate, sandy loam	Alluvial fan over basalt
B.	Cow Loan	Brown and chocolate, red loam, fairly heavy	Alluvial flat over basalt
C.	Kimming Hill	Fine, sandy, brown loam	Glacial sand and gravel over basalt
D.	Hay Knowes	Red and chocolate, gravelly loam	Glacial sand and gravel over basalt
E.	Lambing Field	Dark brown, light loam	Glacial gravel and scree over basalt
F.	Hill Field	Chocolate and brown loam	Thin boulder clay over basalt
G.	House Park	Chocolate and brown loam	Boulder clay over basalt
H.	Glen	Brown loam	Boulder clay over acid andesite
I.	Caerketton	Dark brown, peaty soil	Scree and glacial gravel over acid andesite
J.	Glen	Cinnamon brown, fluffy loam	Thin glacial gravel over boulder clay, overlying basic andesite
K.	Seafield	Fine, brownish loam	Boulder clay, over sandstone (Carboniferous)
L.	House Park	Chocolate and reddish, compact gritty clay	Boulder clay over basalt

Mechanical Analysis.

The results of the mechanical analyses of the soils, made according to the method of the Agricultural Education Association (12), are given in Table II.

The highest percentage of clay is in sample L, Table II, which is the boulder clay, and generally the soils on boulder clay have the higher clay content, but even the clayey content of the soils on the glacial gravel may be fairly high.

Table II.

	A	B	C	D	E	F
Coarse sand	22·86	14·20	36·65	25·02	15·77	25·99
Fine sand	32·29	30·16	32·29	35·63	26·67	31·52
Silt	11·58	16·05	6·28	8·00	11·86	9·00
Fine silt	12·89	15·79	7·18	9·48	10·74	10·64
Clay	10·41	14·07	7·50	11·40	14·88	9·86
Moisture	2·78	2·59	3·79	2·53	4·41	4·84
Ignition loss	7·11	7·16	6·39	7·43	15·91	8·89
Total	99·92	100·02	100·08	99·49	100·24	100·74

	G	H	I	J	K	L
Coarse sand	24·89	17·31	20·86	16·16	26·83	23·88
Fine sand	31·38	21·70	26·44	28·56	28·52	27·54
Silt	11·44	8·82	11·28	8·12	12·05	9·00
Fine silt	12·16	13·36	17·40	9·12	9·92	12·56
Clay	12·82	17·66	11·40	13·08	7·96	20·08
Moisture	2·43	3·79	2·64	5·03	4·91	2·84
Ignition loss	5·62	17·66	9·85	19·35	9·02	4·06
Total	100·74	100·30	99·87	99·42	99·21	99·96

E is such a soil and should be compared with C, also taken from a gravel-spread, but in this case the clay content is low while the coarse sand fraction is high. The soils on the glacial sand gravel-spreads are very variable in texture because of the varied nature of the deposit.

In all cases the percentage of the fine sand fraction is fairly high, and since this fraction was used for the mineralogical analysis, it may be taken as typical of the "fine earth" fraction of the soils.

Mineralogical Analysis.

In the mineralogical analysis the writer has followed the method detailed by Hendrick and Newlands(4). The fine sand fraction of the mechanical analysis was taken and the minerals present separated into three groups according to specific gravity, the heavy liquid used for the separation being bromoform. The first separation divides the mineral grains into two fractions, one of specific gravity greater than 2·9, the other less than 2·9. The latter fraction is then separated into two groups, one of specific gravity greater than 2·6, and the other less than 2·6. Resort is made to the electro-magnet to remove flaky minerals like biotite and weathered ferro-silicates which remain in the higher group by reason of their habit or lowered density through weathering, though their density when fresh would put them in the heavy group.

The three fractions, according to Hendrick and Newlands are characterised by ferro-silicates, quartz and orthoclase, though attention is drawn to the difficulty that may arise in obtaining a clean separation of the latter group (see Pl. I). In the present work the writer found that the percentage figure for the orthoclase group does not represent the

proportion of orthoclase present, the major part of the group in all cases being made up of light quartzose material and weathered plagioclase felspar, and in a few cases some rock fragments. Orthoclase and microcline are minor constituents. This difficulty is caused by the fact that the difference in specific gravity between quartz (2·65) and orthoclase felspar (2·56) is only 0·9, while the plagioclases range in specific gravity from 2·62 to 2·75.

The coarse sand and the silt fractions were also examined for comparative purposes, but no attempt was made to separate the minerals.

Table III. *Results of analyses of fine sand fractions, given as percentages.*

No.	Quartz group	Orthoclase group	Ferro-silicate group
A	88·3	6·3	5·4
B	78·7	6·7	14·6
C	75·9	6·6	17·5
D	78·0	8·3	13·7
E	86·3	7·1	6·6
F	77·2	7·9	14·9
G	89·2	4·3	6·5
H	89·7	2·1	8·2
I	85·6	10·2	4·2
J	81·7	7·1	11·2
K	75·9	6·6	17·5
L	89·5	2·2	8·3

In Table III are given the percentages of the three groups for the soils examined, and the figures should be read in conjunction with Table IV which gives the minerals present other than quartz. In this table the numbers set vertically indicate the frequency of occurrence of the minerals in the soils, 1 meaning most common, 2, 3, 4, etc., indicating decreasing frequency.

Table IV. *Mineral suite—other than quartz.*

	A	B	C	D	E	F	G	H	I	J	K	L
Plagioclase	2	2	2	3	3	2	1	2	2	3	2	2
Orthoclase and microcline	4	4	4	5	5	3	5	6	7	6	4	5
Iron oxides	1	1	1	1	1	1	2	1	1	1	1	1
Biotite	5	5	5	4	4	5	4	7	6	4	5	6
Augite	3	3	3	2	2	4	3	3	3	2	3	3
Hornblende	6	9	—	—	8	9	7	10	12	9	7	9
Apatite	8	—	10	9	11	8	9	8	8	7	—	8
Garnet	9	10	7	6	7	10	8	5	5	5	6	7
Zircon	7	7	6	7	6	6	6	4	4	3	8	4
Tourmaline	11	11	8	10	10	11	11	12	11	8	11	10
Rutile	10	—	—	—	13	—	12	11	9	—	—	12
Chlorite	—	6	—	—	9	7	—	—	—	—	9	11
Glauconite	—	—	—	—	—	12	—	—	—	—	—	—
Enstatite	—	—	—	—	—	13	—	—	—	—	12	—
Rock fragments	12	8	9	8	12	—	10	9	10	10	10	13

Description of Minerals in the Fine Sand Fraction of the Soils.

Though there is a great variety of material in the geological sense from which the soils are derived it is interesting to note that there is little variation in the *shape* of the constituent minerals in all the soils excepting those forming on the screes. That is to say, the sub-angular to angular shape of the mineral grains of soils lying on the boulder clay is not very dissimilar to the shape of the grains of the soils on the fluvio-glacial material. The grains in the soils lying on alluvium are more rounded but not markedly so. This would indicate that the alluvial material cannot have travelled far, and, as will be shown later, the mineral suite indicates that it has practically the same mineral composition as the boulder clay.

Quartz is the commonest mineral present in all the soils. It generally occurs in rather sub-angular grains, but the conchoidal fracture gives rise to angularities. This is most marked in the soils on boulder clay, and also on fluvio-glacial material, but in the soils found on alluvium the grains tend to be more rounded. Inclusions are quite frequent, the commonest being iron oxides, but fluids are also present, and in several cases needles of rutile were noted. Frequently re-growths can be seen on the grains.

Plagioclase felspar. This mineral occurs in all the soils examined and, apart from quartz, is the most plentiful. It is generally weathered, but fresh grains also occur, and then they are quadrate in shape. The weathered fragments are turbid from decomposition.

Orthoclase felspar is not nearly so common as plagioclase, but it is present in all the soils examined. It occurs in quadrate grains, which are generally sub-angular to angular, but in the soils derived from alluvium they tend to be more rounded. It is unweathered. Allied to orthoclase in chemical composition but differing from it in habit is microcline, which is also present in all the soils but in a very subordinate amount. It is very fresh.

Iron oxides, after plagioclases, are the commonest minerals, magnetite, ilmenite, haematite and limonite all being present. It is difficult to distinguish magnetite from ilmenite microscopically, and only grains showing ilmenite weathering to leucoxene were taken to be ilmenite. Leucoxene is rather common and is present in all the soils. Magnetite is the most frequent oxide and the grains are often faceted. Both limonite, the commonest oxide after magnetite, and haematite occur in earthy form.

Biotite occurs in rather ragged plates, which are generally bleached and is rarely fresh. Rounded grains also occur in the boulder clay. It occurs in all the soils and is dark brown in colour, with spots of iron oxide.

Augite. After the iron oxides, augite is the commonest mineral present. It occurs in irregular prismatic grains of variable size. Its colour is usually greenish, but the titaniferous variety is also present, though in subordinate amount, and then the colour is brownish.

Hornblende is present in all the soils save C and D. It occurs in prismatic grains generally small in size and green in colour. It is fresh.

Apatite is infrequent and was not found in all the soils. It occurs in small prismatic grains, often rounded. Its infrequent occurrence in the fine sand fraction may be accounted for partly by its habit and partly by its ready solubility, which would tend to make it more common in the fine grades.

Garnet occurs in all the soils. It is generally colourless, but a pinkish-brown type also occurs. It is angular to sub-angular in shape and is granular.

Zircon is common in all the soils. It is generally prismatic with pyramidal terminations, but ovoid grains also occur. It is usually colourless, but brownish varieties occur. Inclusions are common.

Tourmaline. This mineral is present infrequently in many of the soils. Brown is the commonest colour, but green-brown varieties also occur. Its form is prismatic, but the terminations are irregular. It is very fresh.

Rutile is also infrequent. It is deep brown in colour and is irregularly prismatic and angular.

Chlorite occurs in certain of the soils as rounded grains, green in colour.

Glauconite. In soil F a green amorphous mineral was isolated, which from its refractive index and optical properties suggest glauconite rather than chlorite. Glauconite does not seem to be common in Scottish Carboniferous sandstones and has not been found there by Bosworth (13). Cayeux (14) has suggested that it may be an authigenic mineral in arable soils.

Enstatite was found in only two soils and is rare. It is colourless and prismatic in habit.

Rock fragments are matrix fragments of the local lavas, in certain cases determined as rhyolite, in others as basalt.

The soils in general contain a large proportion of ferro-silicate minerals

which are comparatively fresh, that is, only slightly altered chemically, and having a large reserve of bases.

Minerals and Plant Food.

In all the soils examined above, orthoclase, microcline and biotite were found to occur. Now those are among the most important minerals containing potassium, and their amount and condition of freshness must have much to do with the amount and availability of the potash necessary for plant food. Of the Boghall soils then it may be said that potash is present in fair amount, but that since biotite is the only potash-bearing mineral present in a weathered state the availability of the potash may be low.

In the majority of the soils examined apatite was detected, although in small amount, and it may be present in the other soils, since it is readily soluble. It is the only phosphate-bearing mineral in the soils.

The main lime-bearing minerals are plagioclase, augite and hornblende. The plagioclase occurs as quadrate grains which are mainly weathered, although fresh grains do occur. Augite and hornblende are generally fresh. Garnet, though lime bearing, is not easily decomposed, and is fresh.

Soils on the Scree Material.

Sample I of Table IV is a soil taken on the hill-wash of the acid belt of rock on Caerketton Hill, but from the nature of the mineral suite it will be seen that glacial material must be intermixed. The percentage of the ferro-silicate group is lower than that of the soils derived from drift material, but the mineral suite is not very different. In a soil lying on the top of Caerketton Hill the heavy residue was too small to be measured, but zircon, garnet, rutile, and tourmaline were present along with augite and iron oxides. The main mass of the fine sand fraction was made up of rock fragments (rhyolite) with quartz grains. This would indicate that the ice had swept completely over the hills and that a wash of material had been laid down and then mostly removed by denudation.

In a soil formed on the scree to the south of Allermuir Hill the fine sand fraction was found to consist of about 60 per cent. of quartz, the remainder being mainly composed of rock fragments, plagioclase, iron oxides, augite. The minerals were all heavily stained with iron oxides. In a similar soil on Caerketton Hill the same constituents were present in the same condition but the proportions were different, the quartz percentage being much higher.

The minerals of the soils on the screes are very angular and the soils differ from those on the drift material in having a large content of rock fragments.

Relation of Soils to the Drift Material.

Sample L in Table IV is an analysis of the boulder clay, as found in the glen. It represents horizon C of the surface soil, sample G. From the mineral suite it will be seen that its content resembles the content of the surface soil, and apart from slight differences in frequency, the content of all the soils lying on glacial drift (whether boulder clay or fluvio-glacial material) and alluvium is practically identical. This indicates a similarity in origin. The interesting question of the origin of the matrix of the boulder clay is also raised.

The alluvium from its situation cannot have travelled far, and both the physical characters of the grains, which are sub-angular rather than rounded, and the mineral suite point out its local origin by denudation of the boulder clay. It is mainly over boulder clay that the stream runs.

Of the minerals in the boulder clay the predominant, apart from quartz, are iron oxides, plagioclase, biotite, augite. All these minerals are found in the local lavas as are also hornblende, apatite and chlorite, which occur in subordinate amount in the boulder clay. Zircon also occurs in the local lavas, but the zircons in the soils are probably too numerous, and in many cases too large to be local. Of the minerals present, which can definitely be said not to be local, are the following: zircon, garnet, tourmaline, rutile, enstatite. These minerals may be formed from the disintegration of boulders of Highland rocks carried from the north-west by the ice, but they may also be derived from the sandstones of the Carboniferous formation to the north-west of the Pentland Hills, since Bosworth (13) has found them present in Carboniferous sandstones. The matrix of the boulder clay may, therefore, be said to be mainly made up of material derived by the disintegration of local rocks. The soils of the farm may be definitely linked to the underlying rocks through the boulder clay. The character of the erratics may serve only as an indication of the direction from which the ice has come and may not indicate the nature of the drift matrix.

The geological nature of the materials from which the soils of the Experimental Farm have been formed, has had a marked effect on the texture of the soils. This is well seen in the great variability of the soils lying on the gravel-spread to the south of the road. The boulder clay

soils are the heaviest while the alluvium gives rise to soils of a silty character.

In comparing the mineral suite of the soils with those obtained by Hendrick and Newlands (4), it will be seen that while the state of freshness and general physical character of the grains agree with those derived from the Old Red Sandstone formation, the mineral suite is more allied to that of the soils on the Carboniferous formation. This may be partly explained by the lithological variation in the Old Red Sandstone formation, in that the Old Red Sandstone of the farm area is composed of lavas, while in the areas examined by Hendrick and Newlands sandstone and flagstones predominate.

Summary.

1. An account is given of the geology and mineralogy of the soils of a small area characterised by diverse rock groups, which are mainly covered by glacial drift. The topography is also varied. The soils are mainly derived from boulder clay, glacial sands and gravels, and alluvium, the remaining soils being formed on screes and hill-wash. The underlying rocks are lavas of Old Red Sandstone age and sandstones and shales of Carboniferous age.

2. A similarity in mineral content of the soils on glacial material and alluvium is shown. All these soils have a high content of fresh ferro-silicates. The soils on the screes and hill-wash are characterised by their content of rock fragments and iron oxides, but minerals from glacial material are also present, though to a minor extent.

3. The soils on the drift material contain potash, phosphate and lime-bearing minerals.

4. The varied nature of the parent materials has given rise to varied textures in the soils. The soils on the boulder clay are the heaviest, while the soils on the fluvio-glacial material are very variable.

5. The mineral content of the matrix of the boulder clay is similar to that of the local rocks, only the rarer minerals being derived from external sources.

Acknowledgments.

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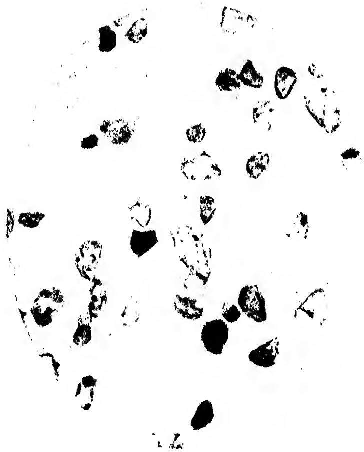

Fig. 1. Quartz and plagioclase grains. (Ordinary light. × 35 diam.)

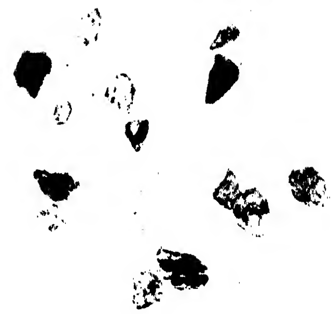

Fig. 2. Orthoclase grains. The dark grains are rock fragmen (Ordinary light. × 35 diam.)

Fig. 3. Heavy residue. Minerals shown are iron oxides, augite, hornblende, zircon, apatite, garnet and tourmaline. (Ordinary light. × 35 diam.)

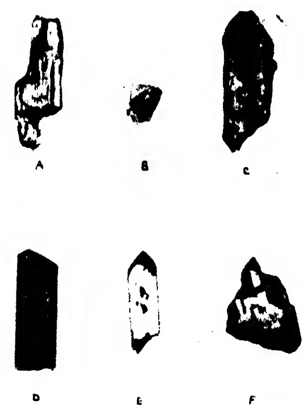

Fig. 4. A. Augite. B. Biotite. C. Hornblende. D. Tourmaline E. Zircon. F. Garnet. (Ordinary light. × 90 diam.)

Photomicrographs of minerals from a soil derived from boulder clay.

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THE DISPERSION AND MECHANICAL ANALYSIS OF HEAVY ALKALINE SOILS.

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(With Four Text-figures.)

I.

1. *The dispersion of heavy alkaline soils.* The principal problem in mechanical analysis lies in the satisfactory dispersion of the soil in order to ensure proper separation of the clay fraction. Physical treatment, acid and hydrogen peroxide, and various alkalis are all used for this purpose, but it appears to us that there is one aspect which requires bringing up to date in view of the work in recent years dealing with exchangeable bases, and that is the application of what is actually known to take place during the treatment of the soil prior to sedimentation. It is, for example, known (and additional evidence is given in a later section of this paper) that sodium clay is more finely dispersed than calcium, ammonium or potassium clay. If, then, the object of the clay determination is to give a figure for the largest proportion of fine particles obtainable under conditions which are as severe as possible without decomposing the mineral part of the soil, the treatment prior to sedimentation should be such as would give a complete conversion of the clay into its sodium compound whatever the original bases may have been. The logical procedure for this would be treatment with an alkaline solution of a sodium salt, applied either directly or after a preliminary acid treatment, which would displace the bases and substitute hydrogen.

In order to investigate this question, the mechanical composition of representative Sudan soils has been determined down to limits much smaller than 2μ using Robinson's pipette method (1), and a number of observations have been made in the course of the work which bear on dispersion problems in general.

The following are the soils which have been used:

Table I.

Description of soil	Nature	Mechanical analysis of fine earth: Clay	Silt	Fine sand	Coarse sand
A. Badob from Gezira	Very plastic	63	19	12	6
B. Alluvium from Kassala	Plastic	75	20	5	0
C. Soil from red sandstone, southern Gezira	Very slightly plastic	56	10	13	21
D. Red sub-soil from Mongalla	Non-plastic	78	17	4	1

The soils were sieved through a 1 mm. sieve, oven dried[1], washed with water to remove salts and then shaken in a reciprocating machine with 0·2 per cent. sodium carbonate for six hours and transferred to the sedimentation cylinder, where the concentration of the sodium carbonate was reduced to 0·05 per cent.

Fig. 1 gives the results obtained, the summation percentages being plotted against the logarithm of the diameter of the particles expressed in $\mu\mu$ in order to avoid negative logarithms.

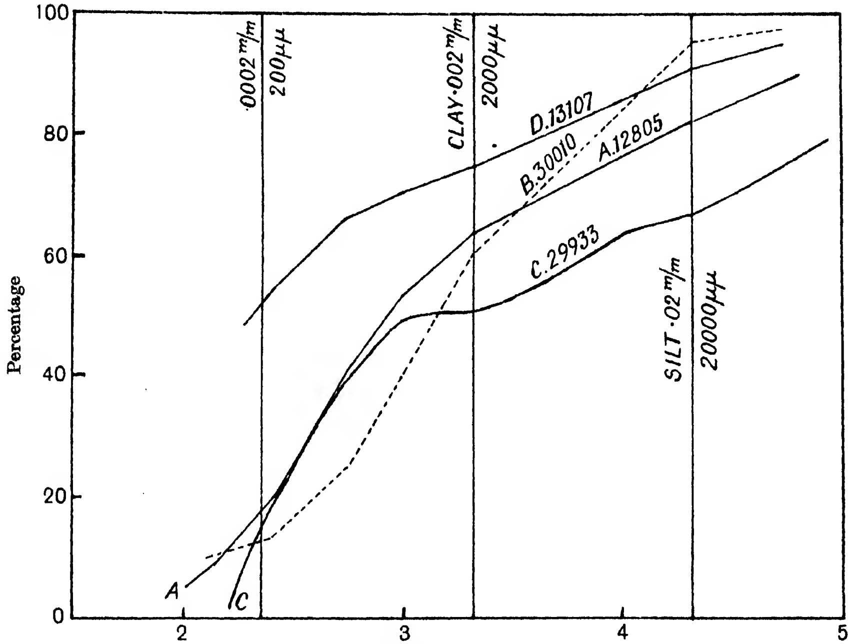

Fig. 1.

The results are particularly interesting in view of the physical properties of these soils of which the order of "clay-like" properties (*e.g.* plasticity) is *A*, *B*, *C*, *D*. There is, therefore, no connection between these properties and the proportion of the finest fraction as determined by a single sedimentation. It is, however, quite possible that a true indication of size distribution cannot be obtained in this way, as experiments with the supercentrifuge (2) showed that the clay fraction of such a soil as *A* is composed almost entirely of "ultra clay" particles probably less than

[1] Oven-drying was found to have no appreciable effect except with hydrogen soil.

$0{\cdot}2\mu$ diameter. The curve only shows about 20 per cent. of the clay in this form, and it is suggested that the only method of ascertaining the true proportion of fine particles under conditions of complete dispersion would be a decantation method. It is hoped to investigate this shortly. Meanwhile the distribution results obtained by a single dispersion may be regarded as empirical but as giving results of considerable interest.

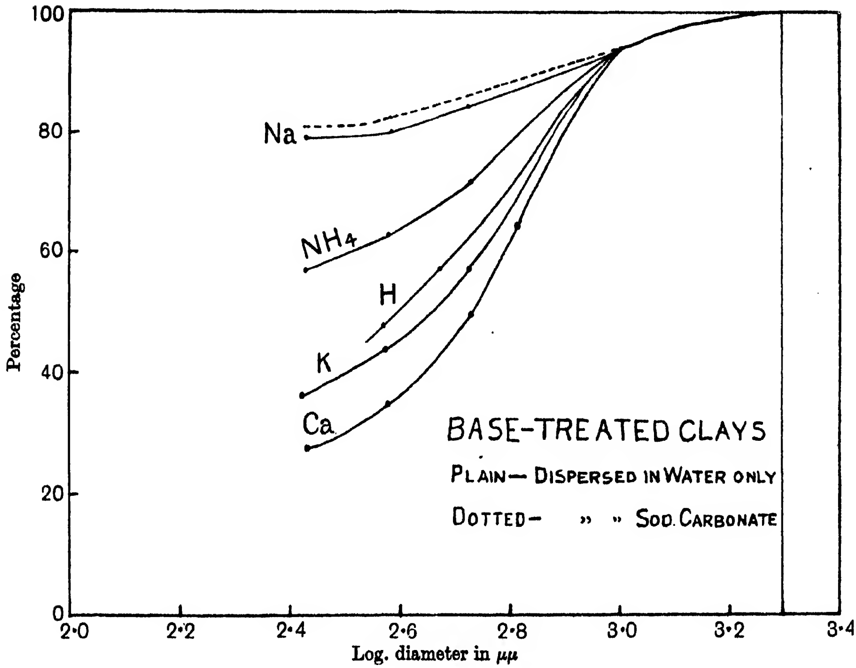

Fig. 2.

2. *The dispersion of soils containing different exchangeable bases.*

(NOTE. This investigation was completed before we received the recent paper of Thomas (3), but there is so little overlapping that we have left this paragraph in its original form.)

The use of an alkaline medium (ammonia or sodium carbonate) to aid dispersion introduces a complication due to the partial or total exchange of the bases in the soil for ammonium or sodium. We have attempted to obtain results for soils containing only one base, by preparing the base-saturated soil[1], separating and washing it with the aid

[1] For this purpose 50 gm. of acid treated soil were percolated with one litre of a normal solution of the appropriate chloride.

of the supercentrifuge and suspending it in water only. The specific resistance of the 1 per cent. suspensions used was always greater than 20,000 ohms.

The results for soil *A* containing hydrogen, potassium, ammonium and sodium respectively are shown in Fig. 2, the 0·002 fraction being put equal to 100. It is difficult to include the coarse material when using the supercentrifuge.

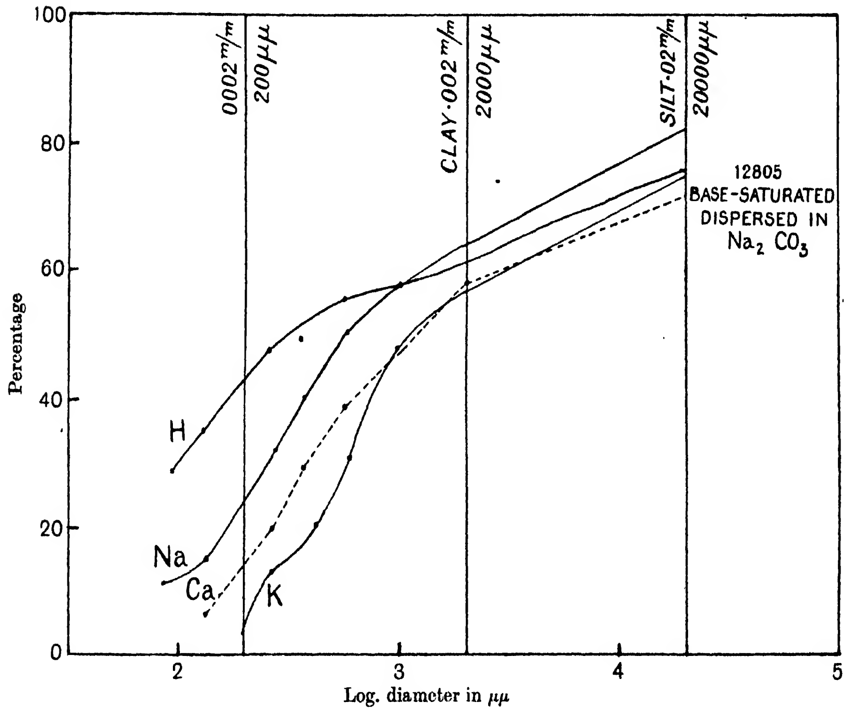

Fig. 3.

These results are surprising and not in accordance with general experience obtained in working with purified clays, as it would class hydrogen and calcium together as being much less dispersed than sodium, whereas the stability of a suspension of purified hydrogen clay appears much nearer that of sodium than calcium. This was noticed some years ago (4) when it was found that for a clay purified from a soil similar to *A*, the stability of the hydrogen clay was actually greater in water than in 0·05 per cent. sodium carbonate solution. Part of this inconsistency may be due to the relative difficulty of dispersing the

different soils in water rather than to differences in the actual proportion of fine particles which would be obtained if the dispersion was complete instead of limited to a single treatment.

Fig. 3 shows the results obtained if the base-treated soils are procured without acid pre-treatment and dispersed in sodium carbonate solution. If the action of the sodium carbonate were to convert completely the soils into sodium soil, these curves should be identical: the differences between them may indicate the extent to which it has failed to do so at one operation. If the liquid could be removed and replaced by fresh sodium carbonate solution, complete replacement by sodium would doubtless take place in time. That it is more readily brought about by treating the base-treated soil with acid to remove all bases and then dispersing with sodium carbonate is shown by the figures for the base-treated soils of curve II when dispersed in sodium carbonate solution instead of water. The figures for the percentage of fine particles were then as follows:

Table II. *Dispersion of base-treated soils followed by acid-treatment and dispersion in sodium carbonate.*

Diameter mm.	Base originally present				
	Ca	K	Na	NH_4	Original
0·002	57·0	61·0	61·2	61·0	61·5
0·001	53·7	57·3	57·8	57·2	57·6
0·0006	52·1	54·8	56·6	54·8	55·7
0·00035	51·5	50·5	54·5	50·8	50·5
0·00025	47·7	47·8	51·5	48·4	48·2

These give nearly coincident distribution curves, due to the fact that the clays were all converted into the sodium compound by the treatment.

A few experiments were made with specimens of sodium and ammonium soils which had not been allowed to dry in order to provide data bearing on the relative merits of ammonia and sodium carbonate as dispersing agents. The results were as follows:

Table III.

Diam. mm.	0·002	0·001	0·0005	0·00026
Na	100	94	84	79
NH_4	100	94	71	57

This indicates the superiority of sodium carbonate over ammonia for dispersion, and is confirmed by the results for mechanical analysis given in a later paragraph.

3. *The bearing of the form of the dispersion curves on the size limit for clay.* In choosing the limits for the clay fraction, it is of great advantage

to make the selection so that a slight alteration in the time or temperature of sedimentation does not exert a marked effect on the result. This effect is best seen by an examination of curves connecting time of sedimentation directly with percentage of fraction, any logarithmic form obscuring the relationship. Fig. 4 shows the results for four different soils assuming the sedimentation to be carried out at 17·5° at which temperature the 0·002 fraction requires eight hours at 10 cm. It will be

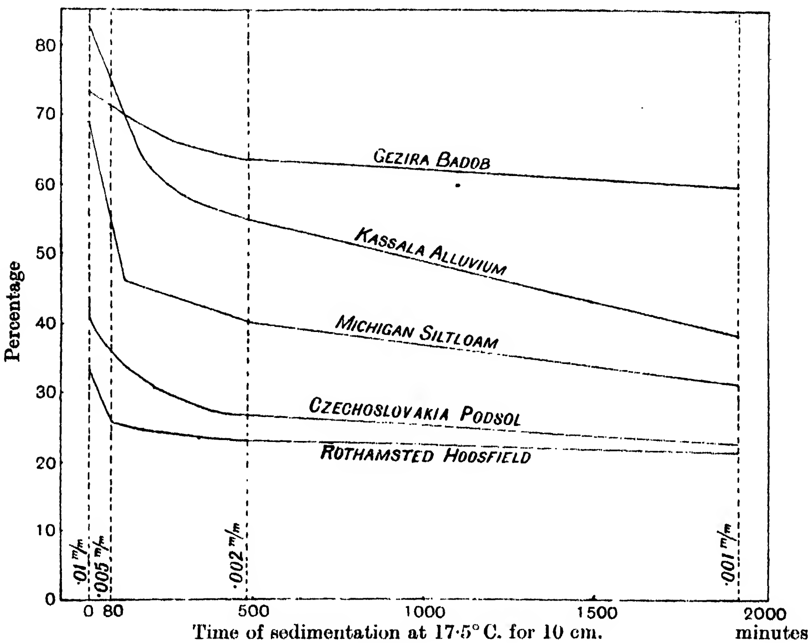

Fig. 4.

seen that a small variation in the time after about six hours (corresponding to a diameter of 3·5μ) affects the amount of material left in suspension only very slightly, so that the limit of 2μ is satisfactory from this point of view without involving an unduly long time. The effect of temperature can also be seen from the curves. This may be allowed for by altering either the height or the time of sedimentation, and we prefer the latter. For the 2μ fraction the time in minutes for 10 cm. sedimentation is equal to 47710 × viscosity of water at the working temperature, which in these laboratories is often as high as 35°. The temperature effect, however, is almost negligible in most cases if the limit is 2μ: thus if the temperature at which the analysis should have been made were 35° C.

instead of 17·5° C., the time of sedimentation ought to have been reduced to 5½ hours, but the difference for the soils in Fig. 4 for eight hours at 17·5° and eight hours at 35° (equivalent to 11·6 hours at 17·5° C.) is insignificant except in the case of the Kassala soil. If a limit higher than 2μ (*e.g.* 5μ) were adopted, the difference would be important in all cases.

The times of sedimentation for the work here recorded have been corrected for temperature in all cases.

For the decantation method, provided the temperature is above 20° C., it is sufficient if the two or three last decantations are made at the corrected time. If, however, the temperature is below 20°, the correction (if required at all) must be made from the beginning. It should be noted that if the time of sedimentation is to be fixed in accordance with Stokes' law, calculation shows that it should be not eight hours at 10 cm. and 20° C., but 7·5 hours: eight hours would be correct for a temperature of 17·5° C.

II.

4. *Mechanical analysis*. Since the previous communication from these laboratories (5) on mechanical analysis was published, many changes have been proposed in technique and an important step has been taken towards the standardisation of a single method. This method now bearing the imprimatur of the International Society of Soil Science is, with slight modifications, that of the Agricultural Education Association described fully in a previous issue of this *Journal* (6). The Report discussed the general principles on which a system of mechanical analysis should be based and the extent to which the variety of procedure used in the preliminary operations affects the results.

That this method has been endorsed by the International Society is of great importance, although, as far as we have been able to ascertain, it has not yet come into common use in laboratories where large numbers of analyses have to be undertaken, so that little experience is available as to what is involved in large scale work such as arises, for instance, in soil surveys.

But in the numerous discussions which have taken place (such as at the Meeting at Rothamsted of the First Commission of the International Society in 1926) one point has been frequently stressed, and that is the impossibility of evolving a method of mechanical analysis of which the details will be the same for all soils. Peats, heavy alkaline soils, laterites, ferruginous soils and terra rossa have been mentioned as examples of soils which might require the introduction of modifications into the

International method, and until it has been in practical use for some time in various parts of the world, it is difficult to say what such modifications should be.

The most important soils of this country being of a heavy alkaline type not commonly met with in Western Europe, we have examined specially the method with respect to such soils, comparing it with the Sudan method which has not yet been superseded for ordinary work, in the course of which about 2000 analyses are made a year.

5. *Soils used for comparative analysis.* Although we have been specially concerned during the last few years with the "badob" soils of the Gezira, the range covered by these investigations has been very wide and the following table gives their characteristics:

Table IV.

Origin	Type	Description	Calcium carb. cont.	Organic matter	Remarks
1. Sudan Gezira	Badob loess	Heavy alkaline	5 %	1 %	Salts and gypsum
2. Sudan Gezira	From sandstone	Red clay loam	None	Low	Low in salts
3. Sudan Kassala	River Gash alluvium	As (1) but less so	,,	,,	Low in salts
4. Sudan Mongalla	Fine sub-soil	Reddish brown clay soil	,,	,,	Fine but non-plastic
5. Czechoslovakia	Podsol	Yellow loam	,,	,,	Low in salts
6. Czechoslovakia	Rendzina	Black loam	6 %	5 %	—
7. Rothamsted	Hoosfield soil	Clay loam	1 %	High	—
8. Michigan	Silt loam	Black Cornbelt soil	None	,,	1·5 % humus
9. Sierra Leone	Lateritic gravel	Reddish brown soil	,,	—	—

6. *The Sudan method.* The method is carried out as described previously, but two emendations should be made. Where the centrifuge is used (which is very rarely in these laboratories), the radius to be considered should be from the centre of the machine to the centre of the column of liquid, and not to the top of the column as stated. The radius for the centrifuge there referred to should therefore be 15 cm. and the time should be 4 minutes at 840 r.p.m.: this would usually have to be reduced owing to the necessary temperature correction dealt with in a later paragraph.

The other alteration relates to puddling. The procedure previously published indicated that a single puddling after the third or fourth decantation would be sufficient. We have however met with a number of soils, notably those with a high ratio of exchangeable calcium to sodium, in which more than one puddling is required (in one case 16 were necessary) so that it is advisable towards the end of the separation,

when only a little clay appears to be left, to puddle again when it will be seen if more dispersion takes place. If so, puddling should be continued at each remaining decantation.

One important detail of the sedimentation procedure requires correction. Following Atterberg, in this laboratory the silt fraction has been separated from a 10 cm. column in 7·5 minutes. At 17·5° this corresponds to a diameter of 0·016 mm. and the time must be reduced to 4·8 minutes at 17·5° for 0·02 mm. This change will be made here at the end of the present year.

7. *Differences between the methods which have been investigated. Effect of oven-drying.* The Sudan sedimentation differs from the International method (as described by Keen (7)) in the following respects: the analysis is carried out on oven-dried instead of air-dried samples, there is no pre-treatment with acid and hydrogen peroxide, 2 hours' shaking in a reciprocating shaker is used instead of 40 hours in an end-over machine, sodium carbonate is used instead of ammonia and the decantation method is used exclusively.

Clay determinations have been carried out on a number of soils using samples (*a*) air-dried, (*b*) dried at 105° for 16 hours, and (*c*) dried for at least 6 days. The average difference caused by the longer period of drying was 0·4 per cent. and in no case did it amount to 1 per cent.: the actual means for nine soils were 59·5 per cent. for the air-dried and 59·3 per cent. for the oven-dried, both expressed on the oven-dry basis.

As it seemed possible that saline soils might be troublesome in this respect, three soils were selected (from near the west bank of the White Nile) containing over 2 per cent. of water-soluble salts, and these gave the following results:

Table V.

Sample	% clay using air-dried soil	% clay on sample dried for 21 days at 105° C.
35170	66·5	66·2
35171	65·8	65·5
35173	66·9	67·0

It therefore appears that there is no objection to our ordinary procedure of oven-drying before commencing the analysis.

8. *Influence of time of shaking.* Evidence was given in the previous paper to the effect that 2 hours' shaking in the reciprocating shaker was sufficient and this has been confirmed, experiments having been carried out with periods up to 30 hours. The dispersion effected by the rotary shaker is much less effective for a given time and its use would greatly

retard routine work. Thus our reciprocating machine takes 24 bottles which can be changed four times a day, *i.e.* nearly 100 samples a day can be shaken. If the end-over machine (which only takes 10 bottles) is to be used for 40 hours, the rate of working is limited to five a day, so that it would have to run for 20 days to do the work that the reciprocator can do in one.

What may turn out to be a most useful aid to rapid dispersion is the stirring machine described by Bouyoucos (8), 15 minutes in which gives roughly the same result as 24 hours in the reciprocating machine. It would, however, be necessary to have a number of them to do the work of one reciprocator.

9. *Effect of pre-treatment with hydrogen peroxide and acid.* This constitutes the most important difference between the Sudan and the official methods, and was first investigated by us in connection with the work carried out in preparation for the Rothamsted meeting above referred to. Only decantation methods were used, and the following table gives the details for them.

Table VI.

	Acid pre-treatment	Hydrogen peroxide	Deflocculant	Shaking (reciprocating)	Decanting liquid
International method	Yes	Yes	Ammonia	2 hours	Ammonia
Hissink's modification	Yes	Yes	None	None	Ammonia
Sudan	No	No	Sod. carb.	2 hours	Sod. carb.

Table VII gives the results for the percentage of clay obtained by these methods in this laboratory and the average of those obtained in eight other institutions taking part in the comparative work.

Table VII.

Soil	Podsol	Rendzina	Badob
Locality	Zdar	Ceji	Sudan Gezira
% organic matter	0·4	4·6	1·0
% calcium carbonate	0·0	5·7	5·9
International method:			
Khartoum	29·1	27·3	63·3
Mean of eight others	28·5	28·4	63·2
Hissink's modification:			
Khartoum	31·8	27·4	63·9
Mean of eight others	30·0	29·2	63·8
Sudan method:			
Khartoum only	27·0	29·0	63·3

In the case of the podsol, the Sudan method appears to give a low result, although the results from the eight other institutions range from 25·0 to 32·4. It may also be noted that this soil, where the agreement

between the three methods is the worst, is the one in which acid and hydrogen peroxide should produce the least effect.

So far, we have not met with any soils for which treatment with hydrogen peroxide appears necessary, and its omission is of importance in tropical laboratories where it is an expensive reagent which keeps very badly. If (to avoid storage) it has to be purchased locally, the expenditure on it in Khartoum might amount to £50 a year or more.

The question of the omission of acid treatment has long been the subject of controversy. Many workers consider that the use of these drastic methods is most undesirable if they can be dispensed with: there is always doubt in the case of acid treatment as to what materials have been removed, and the amount of organic matter destroyed by hydrogen peroxide varies with different soils.

As it is often stated that dispersion is facilitated by acid pre-treatment, three soils were selected to test this point which were very refractory[1] in that they required for the removal of the clay more than a dozen decantations each accompanied by a thorough puddling. The same soils were then acid treated and again analysed, but the number of decantations and puddlings was substantially the same, and from the results given below it will be seen that no more clay was removed.

Table VIII.

	Percentage of clay by Sudan method			
Soil no.	One puddling	Many puddlings	Acid pre-treatment and one puddling	Acid pre-treatment and many puddlings
30100	66·4	74·6	71·7	75·4
30148	61·6	67·6	56·0	69·1
14172	57·9	63·6	60·2	63·0

The pipette method with or without acid pre-treatment relying on one decantation would of course be specially unsatisfactory in such cases. We have not up to the present met with any soils for which acid treatment appears necessary when using sodium carbonate and the decantation method.

10. *Robinson's pipette method as a substitute for decantation.* Ever since the time-saving method of Prof. Robinson was published, attempts have been made to use it for our heavy clay soils containing considerable percentages of water-soluble salts. That good agreement may be obtained between it and our ordinary method is shown by the results already

[1] These were alluvial soils low in organic matter and water-soluble salts and containing no calcium carbonate or gypsum. The reason for their refractory nature is unknown.

quoted for one soil, but where the proportion of water-soluble salts is high, complete dispersion is not effected in one operation. If the salts can be removed by simply washing the soil on the filter paper (as was suggested to us by Prof. Robinson), this difficulty is overcome and the following results show its effectiveness:

Table IX.

Soil no.	35170	35171	35173
% soluble salts	2·6	2·3	2·3
% clay determined by ordinary Sudan method	63·9	63·5	64·6
% clay determined by pipette method without pre-treatment	4·9	5·7	13·3
% clay determined by pipette method without pre-treatment but after washing	65·2	64·8	66·4

The same result is obtained by the official method as the various operations with acid and hydrogen peroxide provide the necessary washings. The omission of the hydrogen peroxide made no difference.

There remains, however, a class of soils (quite common in this country) containing a considerable proportion of calcium sulphate in the form of coarse crystals of gypsum. It is obvious that these cannot be readily removed by water washing, and so far it has been found impossible to obtain a sufficiently satisfactory degree of dispersion to permit of the use of the pipette method. The following examples show the errors which may be introduced owing to this factor, the ordinary procedure being varied as follows:

Method A. Ordinary Sudan method (no pre-treatment, sodium carbonate as deflocculant).

Method B. Pipette method. Pre-treatment consisted of washing for several days on filter paper. Sodium carbonate as deflocculant.

Method C. Pipette method. Pre-treatment consisted of washing 30 times with water with the aid of the centrifuge.

Method D. Pipette method. Pre-treatment with hydrogen peroxide and hydrochloric acid followed by three washings with water.

Table X.

Soil no.	% water-soluble salts (mainly sodium sulphate)	Gypsum	% clay A	B	C	D
24094	0·085	Absent	47·3	40·1	—	—
24096	0·642	Present	53·9	41·9	—	—
24098	0·466	2·2 %	54·1	42·7	—	—
24100	0·425	6·0 %	54·6	39·2	40·9	36·5
24102	0·334	2·4 %	47·5	39·3	40·1	41·4

So far, therefore, no method has been found which will deflocculate

this type of soil successfully in one operation and the decantation method seems to be essential.

11. *Final comparison between the Sudan decantation and International pipette methods.* Twelve of the soils listed in paragraph 5 were used for this purpose and the results indicate the superiority of the former.

A difficulty in the use of the pipette method involving acid pre-treatment lies in the fact that where the clay is weighed directly, considerable uncertainty exists as to the correction to be applied on account of the change in its composition brought about by the pre-treatment and dispersing agent used.

In the case of these heavy alkaline soils the exchangeable sodium or calcium amounts to about 1 per cent. If no acid treatment is used the weight of the clay left after evaporation will require no correction where ammonia is used as the dispersing agent. Where sodium carbonate is used for this purpose the weight of it contained in the volume of suspension pipetted off must be subtracted from the apparent weight of the clay. If acid pre-treatment is used the clay will combine with the alkali in the dispersing liquid. A purified reactive clay will retain 0·5 per cent. of ammonia after drying at 100°, so where this is used the weight of the residue must be reduced by 0·5 per cent. to allow for the ammonia and the percentage of acid clay so obtained increased by 1 per cent. to bring it to its original base content.

When sodium carbonate is used following acid pre-treatment, the acid clay will be converted into the sodium compound, carbon dioxide being lost. To replace the 1 per cent. of sodium originally present will require 53/23 of 1 per cent. = 2·3 per cent. The weight of the residue will therefore be that of the sodium clay, together with the sodium carbonate taken less the weight of the sodium carbonate neutralised by the acid clay. 2·3 per cent. must therefore be added to the percentage of clay found.

The correction to the final weighing can in this case be easily determined experimentally by taking up the residue with salt and water and titrating the sodium carbonate left. This is then subtracted from the total weight of the residue. For six soils the average correction came to 2·4 per cent. on the weight of the clay as against 2·3 as calculated above. The correction will be less in the case of soils which are originally acid and which therefore are less altered by acid treatment in respect of their exchangeable base content.

These corrections have been made where possible in the table below, those for sodium carbonate being obtained experimentally, and those for ammonia assumed to be 0·5 per cent. on the clay.

Table XI.

			Percentage of clay			
				International pipette method		
Soil no.	Class	Remarks	Sudan method (i)	Using ammonia (ii)	Using sod. carb. (iii)	(i–ii)
29933	Gezira sandstone	Low in salts and bases	56·7	55·1	57·4	1·6
12805	Badob	Low in salts	62·5	56·9	61·8	5·6
21502	Badob	Salts present	62·3	60·2	—	2·1
24100	Badob	Gypsum present	54·6	35·2	—	19·4
14172	Kassala alluvium	Ca/Na high	63·6	51·0	53·9	12·6
30100	Kassala alluvium	Ca/Na high	74·6	64·6	67·5	10·0
30124	Kassala alluvium	Ca/Na high	61·4	49·7	54·3	11·7
30148	Kassala alluvium	Ca/Na high	67·6	58·4	60·8	9·2
13107	Mongalla	Sub-soil	80·0	78·1	—	1·9
35359	Hoosfield	Organic matter high	26·9	21·9	—	5·0
27035	Michigan	Black Cornbelt soil	30·8	28·5	—	2·3
37974	Sierra Leone	Laterite	58·7	56·9	—	1·8

The average difference for the twelve soils which gave low results with the International pipette method is 6·9 per cent. Although therefore there are many soils where the International pipette method gives satisfactory results, there are too many exceptions for its adoption to be regarded as safe for these laboratories. Improvement results from the substitution of sodium carbonate for ammonia in accordance with the observation that sodium clay is better dispersed than ammonia, but a single dispersion treatment cannot be relied upon.

SUMMARY.

1. The form of the dispersion curve obtained by single dispersion treatment below 2μ varies greatly with different soils and with the same soil saturated with different bases. There is no connection between the proportion of very fine material (*e.g.* below $0{\cdot}5\mu$) and other important soil properties. The proportion of the very fine material determined in this way would not, therefore, afford any indication of the "colloid" properties of the soil. If the proportion of fine material were estimated by a decantation method the results might be substantially modified.

2. Under the same conditions of dispersion sodium soil is better dispersed than ammonium. Sodium carbonate should therefore be the best medium for mechanical analysis.

3. No case has been met with where the proportion of clay found is affected to an important degree by the use of hydrogen peroxide.

4. With no soil tried was the number of decantations reduced by

the use of acid pre-treatment; those examined gave the same clay content if sufficient puddlings with sodium carbonate were used with or without acid.

5. No method has been found whereby certain soils can be dispersed in a single operation as is required in the pipette method. In some cases this is due to gypsum, but there are others in which the cause of the difficulty of dispersion is not yet known.

6. In the cases examined sodium carbonate gives a higher result than ammonia when using the International pipette method.

7. For Sudan soils decantation methods appear essential, hydrogen peroxide unnecessary, acid pre-treatment not essential, and sodium carbonate better than ammonia. We are of opinion that the same holds good for many other soils.

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THE PROPERTIES OF HEAVY ALKALINE SOILS CONTAINING DIFFERENT EXCHANGEABLE BASES.

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(With Two Text-figures.)

1. The study of the modification in properties produced in soils by the action of electrolytes has been greatly facilitated by recent investigations on base exchange and on the chemical nature of the clay. The study is of special interest in connection with heavy alkaline soils which (occurring as they do mainly under climatic conditions necessitating irrigation) are more subject than others to reactions between clay and salts, these constituting in fact the most important problem of the irrigation of heavy soils.

It is now well established that clay consists of aluminosilicic acids which, although complex, possess properties characteristic of weak acids insoluble in water. The salts of these acids are also practically insoluble, and are transformed one into another by treatment with solutions containing the appropriate cation, such transformation constituting the phenomenon ordinarily spoken of as base exchange. This phenomenon is not peculiar to soil chemistry: any pair of compounds of which at least two of the four possible combinations of ions are insoluble in water may exhibit it, and as shown by the recent and important paper of Kerr (1), base exchange phenomena follow the laws of chemical dynamics to a degree which admits of no doubt as to the propriety of including them with ordinary chemical reactions. The first step, then, in a systematic study of base exchange would appear to be the study of the compounds of the clay acids with different bases.

2. *The properties of pure clays saturated with different bases.* The following data relate to a series of preparations[1] made from the pure hydrogen clay separated from *badob* soil No. 12805 containing 60 per cent. of clay: this clay is one of the reactive type with a silica-alumina ratio of about 4.

[1] Details of the experimental methods used are collected in an appendix at the end of this paper.

The following properties are tabulated below:

(*a*) The viscosity at 35° of a 10 per cent. suspension containing enough of the appropriate carbonate to make the mixture ·04 normal.

(*b*) The weight of methylene blue absorbed by 100 gm. of clay from dilute solution.

(*c*) The turbidity of 0·1 per cent. suspensions containing the appropriate base in the form of carbonate, except in the case of calcium (where the hydroxide was used), and of hydrogen.

(*d*) The absorbing power of hydrogen clay for different bases using 0·04 normal hydroxide with 0·5 normal chloride.

(*e*) The moisture equivalent expressed on a volume basis.

(*f*) The xylene equivalent on a volume basis.

(*g*) The imbibitional water (*i.e.* the difference between (*e*) and (*f*)).

(*h*) The hygroscopic coefficient over water at 37° for 48 hours.

Table I.

	No. 12805 clay saturated with					
Properties	H	Li	Na	Ca	Mg	K
Viscosity (secs.) ...	12·4	15·4	14·7	—	—	14·7
Dye absorption ...	30	37	39	30	32	31
Turbidity ...	100	50	65	95	95	85
Base absorption (equiv. × 10^4 per gm.)	—	9·0	9·6	19·2	—	11·2
Moisture equiv. vol.* ...	178	262	326	164	177	137
Xylene equiv. vol. ...	35	44	34	33	39	34
Imbibitional water ...	143	218	292	131	138	103
Hygroscopic coefficient ...	90	103	100	84	100	80

* c.c. of water or xylene retained by 100 c.c. of dry soil.

The order of the cations is not the same for all the properties in the table, but this is not extraordinary. The fact that sodium, potassium and calcium clays do not show the same relationship in all their properties may be compared with the (quite different) case of the solubilities of the sulphates of calcium, strontium and barium, which are in the reverse order to that of the hydroxides of the same metals.

Similar full data are not available for other clays, but the following relate to one from a red soil (50 per cent. clay) of a much less plastic kind, the silica-alumina ratio of the acid-treated clay being 2·7.

Table II.

	No. 26088 clay saturated with			
Properties	H	Na	Ca	K
Dye absorption ...	17	22	18	18
Moisture equiv. vol. ...	118	214	128	152
Xylene equiv. vol. ...	37	53	46	41
Imbibitional water ...	81	161	82	91

The figures show similarities to those observed for the other clay, but (with the exception of the xylene equivalent) are all lower in accordance with the general principle relating clay-like properties to the chemical composition as evidenced by the silica-alumina ratio.

3. *The properties of soils saturated with different bases.* It follows from the last paragraph that the effect of the introduction of a base into a soil will depend on (*a*) the nature of the base, and (*b*) the nature of the hydrogen clay into which it enters. Assuming that in these heavy soils all the responsibility for base exchange may be thrown on to the clay (an assumption which is by no means always justified), the amount and reactivity of the clay, as indicated by its silica-alumina ratio, determines the amount of base which the soils can take up. Thus the percentage of replaceable calcium present in four heavy soils washed with calcium chloride solution was as follows, the order following the reactivity of the clay:

Table III.

Soil no.	% clay	SiO_2/Al_2O_3 for clay	% Ca in saturated soil	% Ca calculated on the clay
A 12805	60	4·0	0·95	1·6
B 30100/1	75	3·7	0·80	1·3
C 29933/4	50	2·7	0·59	1·1
D 13107	76	2·4	0·26	0·3

The following table gives the moisture equivalent, capillary rise and percentage of fine material for the above four soils saturated with different bases, and the Atterberg plasticity numbers for the original and sodium soils.

The percentage of fine material is obtained by Robinson's method of mechanical analysis, allowing sedimentation to take place in a 10 cm. column for 14 days: this corresponds to a diameter of ·0003 mm. for the upper limit of particles in suspension. The dispersion results are, however, complicated owing to the employment of sodium carbonate solution (0·05 per cent.) as a dispersing medium which will certainly obscure the effect of the introduction of hydrogen into the clay and perhaps the other bases to a lesser degree. The results are, however, of interest in themselves as showing the effects produced by what may be described as a normal treatment.

The case of soil *C* is important as showing the profound alteration in texture brought about by the introduction of sodium: although this soil contains 50 per cent. clay, its texture as indicated by capillary rise is excellent until sodium is introduced, when it becomes as bad as *A* which was originally much less permeable[1]. The other noteworthy point is the

[1] Judged by the plasticity number, however, it is still superior to *A*.

great alteration in properties brought about by the introduction of potassium: the openness of texture is shown by the low moisture equivalent and high capillary rise.

Table IV.

Soil		Moisture equivalent	Capillary rise	% fine material	Plasticity number
A	H	40·0	70	48·9	—
	Original	42·0	33	20·0	40
	Ca	38·0	90	20·2	—
	Na	59·0	6	31·0	60
	K	30·0	195	13·3	—
B	H	36·2	143	27·2	—
	Original	40·3	63	13·1	25
	Ca	36·6	73	14·9	—
	Na	82·3	2	35·8	56
	K	29·2	45	—	—
*C**	H	26·7	268	43·5	—
	Original	25·9	235	20·3	23
	Ca	23·9	288	18·1	—
	Na	53·2	4	31·9	37
	K	26·0	299	20·4	—
D	H	32·4	—	63·4	—
	Original	32·8	—	54·5	15
	Ca	33·7	—	47·7	—
	Na	36·0	—	60·0	—

* This is practically identical with No. 26088 referred to in Table II.

4. *The effect of replaceable bases on the mechanical properties of soils.* The great amelioration in texture of a soil brought about by the introduction of calcium is due to the differences between calcium and sodium (or hydrogen) clays in that the former is (*a*) less plastic, (*b*) shrinks less, (*c*) attains after shrinkage a lower density, and (*d*) a lower mechanical strength, than the latter. The practical importance of these properties demands therefore a close study of the influence on them of different bases carried out quantitatively as far as possible.

The following table gives the properties as far as determined for soil *A* containing a variety of different bases, the experimental methods being as follows:

(*a*) The *Atterberg plasticity* number.

(*b*) The *shrinkage* which takes place on the drying out of a block of soil containing an amount of moisture equal to its moisture equivalent.

(*c*) The *density* of blocks made without kneading.

(*d*) *The mechanical strength.* In the absence of any suitable machine this was estimated empirically by grinding air-dried blocks of soil with a pestle and mortar.

Table V.

Properties	Soil *A* saturated with							
	Li	Na	Mg	Ca	Original	NH_4	K	H
Plasticity No.	82	60	56	42	40	22	22	20
Shrinkage ...	99	51	33	30	34	27	25	31
Density ...	2·11	2·07	2·01	1·78	2·02	1·79	1·45	1·65
Mech. strength	High	High	Moderate		High	Low	Low	Low

Here again the most striking fact brought out by these results is the position occupied by potassium in the series. The properties of ammonium-treated soil are similar, and one result would be that potash or ammonia fertilisers should have an ameliorating effect on texture in addition to the benefits conferred in the form of plant food.

5. *The varying degrees in which bases are introduced and the properties of soils containing mixed bases.* It has been known since the time of Way and Van Bemmelen that soils had not the same absorbing power for different bases, potassium being absorbed most easily and sodium the least. It is not easy to define the experimental conditions under which such comparisons should be made: it certainly appears that the above statement is not true if applied to clay treated with the hydroxide and chloride of the cation, the composition of the mixture being so adjusted that the alkalinity does not exceed a pH value of about 9. Under these circumstances it has been shown (2) that clay takes up equivalent quantities of sodium, potassium, barium and calcium, but that with more alkaline mixtures the amount of cation absorbed increases in the order given. There is, however, a definite inconsistency in the behaviour of potassium, which in dilute suspension behaves like sodium and in the solid condition like calcium.

One similarity between sodium and potassium clays in dilute suspensions is shown by the flocculating power of the hydroxides for acid clay (for soil *A*), the concentrations required to flocculate a 1 per cent. suspension in 1 hour being as follows (flocculation is defined as having taken place when two-thirds of the clay is sedimented):

NaOH	KOH	$Ca(OH)_2$
0·05 *N*	0·06 *N*	0·002 *N*

On the other hand, we find that both potassium and calcium are absorbed preferentially to sodium: this is shown by percolating the soil with a solution containing equimolecular proportions of the chlorides of the two cations under comparison, washing and determining the replaceable bases then present in the soil.

With soil *A* the results were as follows:

Table VI.

Percolating mixture	Ratio of equivalents of bases left in soil
Ca + Na	Ca/Na = 6
K + Na	K/Na = 6
Ca + K	Ca/K = 0·9

To observe the effect of the proportion of bases on the properties of the soil, soil *A* was percolated with a series of mixtures; after percolation, the soil was washed and examined for capillary rise and moisture equivalent, the amount of replacing cations being also determined. The results are as follows, the ratio of the bases being expressed as equivalents:

Table VII.

Calcium and sodium.

Ca/Na in solution...	All Ca	3	2	1·5	1	0·5	0·05	All Na
Capillary rise ...	210	140	100	77	28	9	4	5
Moisture equivalent	38	—	44	—	51	67	122	96
Ca/Na in soil ...	18	—	—	—	6	2	0·6	0·3

Potassium and sodium.

K/Na in solution ...	All K	1	0·33	0·14	All Na
Capillary rise ...	243	70	24	9	1
Moisture equivalent	27	30	43	62	—
K/Na in soil ...	—	6	2	1	0·1

Calcium and potassium.

Ca/K in solution ...	All Ca	3	2	1	0·5	0·33	All K
Capillary rise ...	128*	147	161	169	231	224	287
Moisture equivalent	35·2	34	31	32	33	29	31
Ca/K in soil ...	—	1·6	0·9	0·88	0·83	0·55	0·2

* These soils were so permeable that the readings were taken after 3 instead of the usual 5 hours.

The introduction of potassium causes a very marked alteration in texture-properties; even with the lowest proportion of potassium to calcium, for example, the soil shows practically no cohesive properties.

6. *Exchangeability of bases for hydrogen.* Since natural soils of course always contain replaceable hydrogen it is of interest to compare the relative ease with which hydrogen and the common bases are mutually exchangeable. This has been attempted in four ways:

(*a*) The most direct method consisted of percolating washed acid-treated soil with a solution deci-normal with respect to hydrochloric acid and the chloride of the base concerned, washing the residual soil on a collodion filter and then determining the replaceable base left.

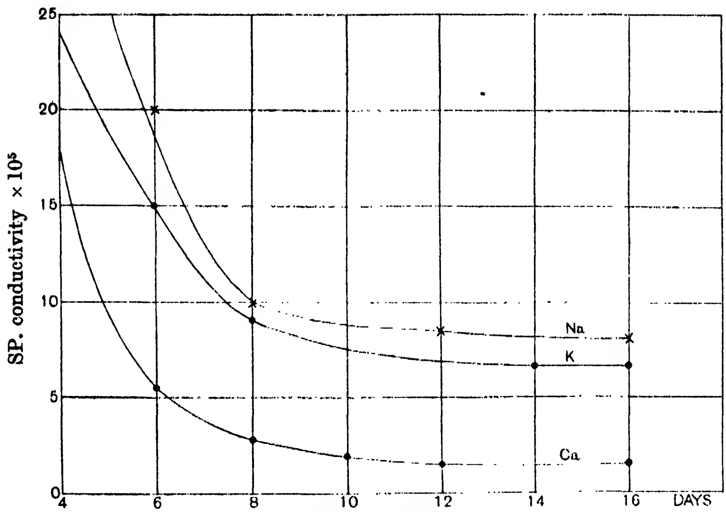
Using soil 12805 the results were as follows:

Table VIII.

	Ca	K	Na
% element left	0·30	0·52	0·19
% as gm. equivalents ...	0·015	0·013	0·009
pH of soil in 1–5 extract	3·81	4·18	4·14

It is interesting to note the comparatively large amounts of base left after a treatment with such a strong acid as $N/10$ HCl.

It is also of importance that the "soil acidity" is not an indication of the amount of exchangeable base the soil contains when the bases are different.



Ordinary dialysis

Fig. 1.

(b) Portions of the same soil were saturated with the same three bases and washed till free from added electrolyte, and the pH of the suspensions determined before and after washing. These measurements give the change in hydroxyl-ion concentration caused by the hydrolysis brought about by washing and consequently indicate the ease with which hydrogen had displaced the base. The results were as follows, the measurements having been made at 34° C. at which Kw for water is 13·6:

Table IX.

	Ca	K	Na
pH before washing	7·92	8·48	9·59
pH after washing	8·63	9·44	9·87
Difference in OH conc. $\times 10^5$	0·9	6·1	8·0

(*c*) Acid-flocculated clay from soil *A* was treated with the appropriate hydroxide and chloride to saturate it with each of the three bases. After washing by decantation the clay suspension was dialysed under fixed conditions as to size of dialyser and frequency of changing the distilled water and the conductivity of the suspension determined daily to follow the removal of electrolyte. After about 10 days very little alteration took place in the conductivity, the final values of which presumably are a measure of the hydrolysis of the clays. The results shown in Fig. 1 indicate the relative extent of this hydrolysis for calcium, potassium and sodium.

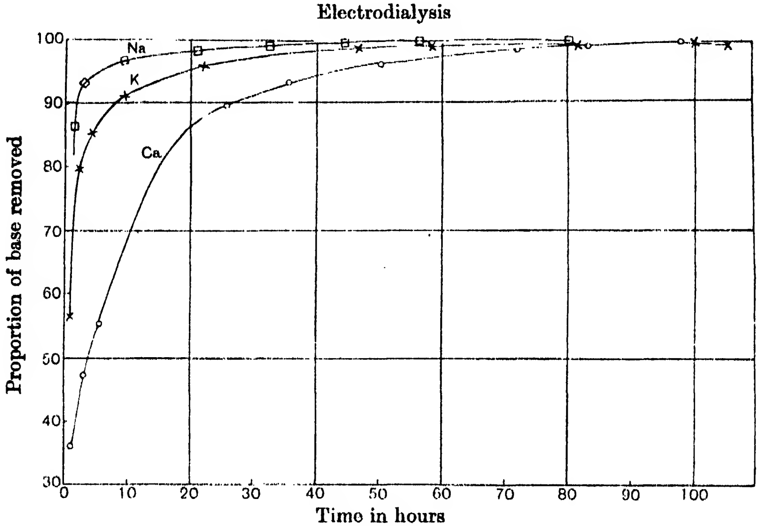


Fig. 2.

(*d*) Washed base-saturated soil was electrodialysed in the cell described by Mattson and the results are shown in Fig. 2. The relative order in which the bases are removed is as in (*a*) and (*b*) above.

7. *The heat of wetting of soils containing different bases.* So much attention has been paid in recent years to the heat of wetting as a soil property, and in particular to its employment as a means of determining the proportion of "soil colloids," that it is advisable to point out that the heats of wetting of soils containing different bases are entirely different from what would have been expected.

It is in general true that a series of natural clays (or soil colloids), probably containing calcium and hydrogen as their main replaceable bases, give heats of wetting in accordance with their general characters,

which again are in accord with their chemical composition (silica-alumina ratio): the same soil, however, treated with different bases, gives different heats of wetting, the values being entirely at variance with other properties. Thus whilst the "colloidal" properties of a sodium soil are far greater than those of a calcium one, the heat of wetting of the latter is actually greater than the former. Figures for a number of soils are given by Pate (3) and have been confirmed by us. The following example shows the nature of the effect and the agreement between Pate and ourselves, the heat of wetting for the untreated soil being put equal to 100.

Table X.

Soil saturated with	Original soil	Ca	Na	K
Oktibbeha soil (Pate)	100	95	69	54
Soil *A*	100	95	84	36

Whilst, therefore, it may be legitimate to use the heat of wetting to compare the properties of soils containing the same exchangeable bases, it is useless when applied to cases when they are different, and in any case gives no indication as to those water-relationships of importance in field studies.

APPENDIX ON EXPERIMENTAL METHODS USED.

1. *Preparation of clays saturated with different bases.* About 200 c.c. of a 2–5 per cent. suspension of acid clay (purified by dialysis as previously described (4)) are treated with the chloride of the base it is desired to introduce, about 1 c.c. of a normal solution being added for every gram of clay taken. The suspension is then made alkaline (pH about 9) with the hydroxide, the mixture allowed to stand 48 hours with occasional shaking and then dialysed until the desired degree of purification has been obtained.

2. *Absorption of methylene blue.* Previously described in (4). The quantities used are 10 c.c. of 1 per cent. clay suspensions, 20 c.c. 0·5 per cent. methylene blue and 5 c.c. of normal sodium chloride, the volume being made up to 80 c.c. (the capacity of an ordinary centrifuge tube).

3. *Viscosity.* This was determined in an Ostwald viscometer using a thermostat at $35° \pm ·01°$. The water value of the viscometer was 10·4 secs.

4. *Turbidity.* Turbidities were measured in a Klett nephelometer using a 0·1 per cent. suspension of hydrogen clay as a standard. As the clays containing different bases are not quite the same colour, it is advantageous to use a red light or colour screen in making the comparisons.

5. *Density.* The density of blocks of soil is determined by a simple balance consisting of a beaker of mercury and an old balance stirrup and pan. From the top of the stirrup projects downwards a piece of strong wire bent into a horizontal ring at the end (about ½″ diameter). This keeps the block of soil immersed. The displacement of the mercury by the ring must be adjusted by the addition of sealing wax until it just floats the stirrup and pan.

6. *Shrinkage.* These figures (calculated for 100 gm. of dry soil) are extrapolated from the moisture-volume curves obtained as described by Haines (5). Extrapolation is necessary as it is not possible to handle blocks as moist as this.

7. *Imbibitional water.* The retaining capacity of 30 gm. of dry soil or 3 gm. of clay for water and for xylene is determined in the moisture equivalent centrifuge: the difference between these values, expressed as c.c. retained by 100 c.c. of dry soil, is the imbibitional water.

8. *Electrodialysis.* The cell, made up with sheets of thick rubber bound with iron clamps, is described by Mattson (6). At 90 volts the current varied between 300 and 20 milliamperes.

9. *Capillary rise.* These measurements are made by packing a column of air-dry soil (passing a 1 mm. sieve) about 30 cm. long into a tube of 14 mm. diameter by a succession of sharp taps on the bench at frequent intervals, the bottom of the tube being closed by a plug of absorbent cotton wool. Water is poured on to the tray in which the tubes are standing and the heights to which the water rises are read off after 3 minutes and again after 5 hours.

10. *Estimation of replaceable bases.* In the absence of an established method suitable for alkaline soils the following procedure is used. 10 gm. of soil are shaken with 80 c.c. of tenth normal ammonium chloride solution and the mixture centrifuged until clear. The liquid is poured off and another 80 c.c. ammonium chloride solution added and again centrifuged. Four treatments are given and the bases required are estimated in the mixed liquid. This method gives lower results than obtained with the use of a normal solution, but as the removal of bases from a soil appears to go on almost indefinitely, the procedure has to be settled empirically.

11. *Hygroscopic coefficient.* 1·5 gm. of clay are left in a saturated space at 37° for 48 hours and the percentage of water determined by drying at 105°. In the series of figures sodium is put equal to 100. Its actual value as determined was 28·3 per cent.

12. *Plasticity number.* This was determined as described by Russell

and Wehr (7). All the results given were obtained in duplicate by one observer whose technique leads to rather high figures: they are, however, strictly comparable.

SUMMARY.

1. The nature of the replaceable base in a clay or soil exerts a profound effect on the physical properties. Clay-like properties are exhibited most strongly in the case of lithium, sodium, and magnesium.

2. The proportion of fine material in a soil (*i.e.* that which remains in suspension in a column 10 cm. high after 14 days) cannot be correlated with other physical properties. Thus a soil of which over 50 per cent. was dispersed to this extent was the least plastic of those examined.

3. A comparison of sodium, potassium, and calcium clays and soil showed that potassium resembles sodium in its chemical relationships as indicated by base exchange, but is very different from it in such physical properties as plasticity and permeability.

4. Using mixtures of one-half normal chlorides of two bases, calcium and potassium are absorbed in equivalent amount while the sodium absorbed is only one-sixth of the amount of either of the other two.

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A PRELIMINARY NOTE ON THE EFFECT OF SODIUM SILICATE IN INCREASING THE YIELD OF BARLEY.

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(With One Text-figure.)

1. Source of Data.

The permanent barley experiment at Rothamsted, of which the first crop was harvested in 1852, contained among others four plots receiving sulphate of ammonia; of these, two plots (2 and 4) received in addition 392 lb. per acre (439 Kg./Ha.) of superphosphate, while also two plots (3 and 4) received sulphates of potassium, sodium and magnesium at rates of 200, 100 and 100 lb. per acre (224, 112, 112 Kg./Ha.) respectively.

The comparison of the yields showed from the first a satisfactory response to the phosphatic manure, but little or no response to the potash and other sulphates.

For the harvest year 1864 and subsequently these four plots were each divided in two, making two series of four plots each. The first series (Series AA) continued the treatment of the previous years, while to the second series (Series AAS) a dressing of sodium silicate was added at the rate of 400 lb. per acre (448 Kg./Ha.). In 1868 the nitrogenous dressing of sulphate of ammonia was replaced by nitrate of soda at the rate of 275 lb. per acre (308 Kg./Ha.).

The remarkable effects of the addition of silicate have already attracted considerable attention, but for lack of analytical data and other reasons it appears that the effect of the addition of silicate has been in some manner misunderstood. This note presents a summary of the results of statistical analyses of the yield data, together with new chemical analyses, which appear to show conclusively that the view previously rejected that the silicate acts by making available to the plant the actual reserves of soil phosphates must be regarded as strongly established.

2. The Effect on the Average Crop.

It was early realised that the plots receiving silicate were yielding considerably heavier crops than those which received no silicate, and

that this effect was especially clear on the plots which received no phosphate. Thus Hall and Morison (1) in 1906 give the following average yields in bushels per acre for the 41 years 1864–1904.

Table I. *Grain in bushels per acre.*

	Plot 1	Plot 2	Plot 3	Plot 4
Series AA (no silicate)	27·3	42·2	28·6	41·2
Series AAS (silicate)	33·8	43·5	36·4	44·5

Table II. *Straw in cwt. per acre.*

	Plot 1	Plot 2	Plot 3	Plot 4
Series AA (no silicate)	16·2	24·6	17·9	25·3
Series AAS (silicate)	19·8	25·8	21·7	27·6

In the absence of phosphate the addition of silicate has increased the yield from 28 to 35 bushels of grain, while in the presence of phosphate the increase is only from 41·6 to 44 bushels. Such results strongly suggest that the effect of the silicate, of whatever nature it may be, is intimately concerned with the phosphatic requirements of the crop. These might be either primarily a matter of soil chemistry, if the effect of the addition of silicates were to make available some portion of the phosphatic reserves of the soil, or primarily a matter of plant physiology, if its effect were to diminish the phosphatic requirements of the plant. Only analyses of the ash can settle this primary question.

It may be remarked at once, however, that the effect can scarcely be ascribed to the sodium rather than to the silicate in the manure added. For all plots receive a large quantity of sodium as nitrate, and plots 3 and 4 in addition receive further sodium, as well as potassium, as sulphate.

3. The Ash Analyses.

Ash is available from samples of grain and straw for nearly every plot in every year. Their phosphatic content is, however, with some exceptions unknown. Hall and Morison give the phosphatic contents of the ash from grain and straw for these eight plots for the harvest years 1903 and 1904. The chemical department at Rothamsted has further supplied me this year with the values for two groups of six years each, namely 1868–73 and 1906–11. These 224 analyses thus supply data for 14 separate years.

For grain the mean content of phosphoric anhydride expressed per cent. of pure ash is shown in Table III.

Table III. *Phosphoric anhydride per cent. of ash.*

	Plot 1	Plot 2	Plot 3	Plot 4	Standard error
Series AA	31·37	35·57	30·51	35·47	0·1735
Series AAS	32·51	35·42	32·74	35·94	

The differences in percentage are comparatively small, but those induced by a dressing of superphosphate are so regular that their statistical significance can scarcely be doubted. In order to test the significance of the smaller effects it is necessary to form an estimate of the standard error of these average values. It is now becoming increasingly realised that a standard error based on the agreement of duplicate chemical determinations is not a sufficient safeguard unless such errors as arise in sampling the produce and the ash can be exhaustively examined. In order to obtain one inclusive estimate we may utilise the close parallelism between plots 1 and 2 receiving no sulphates of potash, soda and magnesium, and plots 3 and 4 which receive them. The differences between these two pairs of plots were therefore calculated for each year, and their deviations from the means of the six early and the six late years respectively provide the estimate of error given in the table, this estimate being based on 40 degrees of freedom.

Judged by this standard there is a clearly significant increase due to silicates in plots 1 and 3, but no significant change in plots 2 and 4.

In Table IV are shown the averages obtained from the analyses of the straw.

Table IV.

	Plot 1	Plot 2	Plot 3	Plot 4	Standard error
Series AA	1·997	3·598	1·830	3·533	0·0399
Series AAS	1·962	3·336	1·816	3·559	—

None of the silicate comparisons can be regarded as significant, except the somewhat large reduction of phosphatic content in the ash from plot 2. There is besides a marked increase on the plots receiving superphosphate and some decrease on those receiving potash.

The data for two years given by Hall and Morison gave indications similar to those of the average of 14 years here presented. These authors, however, write as though the ash analyses showed that phosphoric acid was less abundant in the straw (although somewhat more so in the grain), whereas the analyses in reality only show the proportion of phosphoric acid in a 100 parts of ash. They argue, therefore, that the silicate "gives the plant such a stimulus as enables it to develop more vigorously and obtain more phosphoric acid from the soil"; although on the view that

additional phosphate is not made available, it is difficult to explain why the phosphoric acid in the ash from the grain should not be somewhat decreased. The essential fact that appears to have been overlooked is that the increase of any one ingredient in the ash will *ceteris paribus* tend to diminish the percentage of other ingredients, and this effect will be most clearly apparent when we are concerned with two ingredients such as silica and phosphoric acid which contribute largely to the ash.

We may now turn to the very different picture presented by the total weight of phosphoric anhydride removed in the crop.

4. WEIGHT OF PHOSPHORIC ANHYDRIDE IN THE CROP.

The total ash content of the crop, both in grain and straw, being known, it is an easy matter to calculate from the percentage of phosphoric anhydride in the ash the total content of the crop in this ingredient. This is shown with a standard error, calculated as before, in Table V.

Table V. *Phosphoric anhydride in crop (lb. per acre).*

	Plot 1	Plot 2	Plot 3	Plot 4	Standard error
Series AA	10·50	22·47	10·31	22·40	0·3873
Series AAS	13·82	22·84	15·20	25·67	—

In the absence of all phosphoric fertiliser the crops on plots 1 and 3 have depleted the reserves of phosphoric anhydride to the extent of 10·4 lb. per acre (11·7 Kg./Ha.) in each year. The effect of adding silicates to these plots has been to increase the quantity removed to 14·5 lb. per acre (16·7 Kg./Ha.), or by about 40 per cent. For comparison, the addition of superphosphate, containing, at 16·5 per cent., over 64 lb. of phosphoric anhydride per acre (72 Kg./Ha.) annually, has only increased the amount removed in the crop by 12 lb. (13·5 Kg./Ha.); and when silicate is present in addition this is further increased by nearly 2 lb. In view of these figures it is difficult to avoid the conclusion that, in the presence of silicate, phosphoric acid is made available to the plant, even in plots which have been long depleted in this ingredient without replacement, in very considerable quantities.

5. THE PHOSPHATIC CONTENT OF THE CROP BY DRY WEIGHT.

The quantities of phosphoric anhydride removed in the crop, although striking in quantity and long sustained, are not competent to provide a decisive disproof of the suggestion that the effect of silicate in increasing the phosphate removed is an indirect consequence of a

stimulus to the growth of the plant. Such disproof must be sought in the phosphatic content of the crop expressed as a fraction of its dry weight. For it is evident that if the increased growth of the crop is not due to increased abundance of available phosphate but to some other nutrient or stimulus, the phosphatic content of the plant reckoned as a fraction of the total organic matter present could not be increased, but must, if any change is perceptible, be diminished; whereas, on the contrary, an increase in growth directly stimulated by an increase of available phosphates might reasonably be expected to be accompanied by an increase of phosphatic content in the organic matter. A strict application of this test would perhaps require that the phosphatic content should be reckoned per 1000 parts of dry matter *less ash*; since, however, the absolute amounts of ash, though variable, are not large, only quite negligible errors will be introduced by expressing the phosphoric anhydride in 1000 parts of dry matter.

In view of the importance of this measure we shall give the averages separately for the three periods.

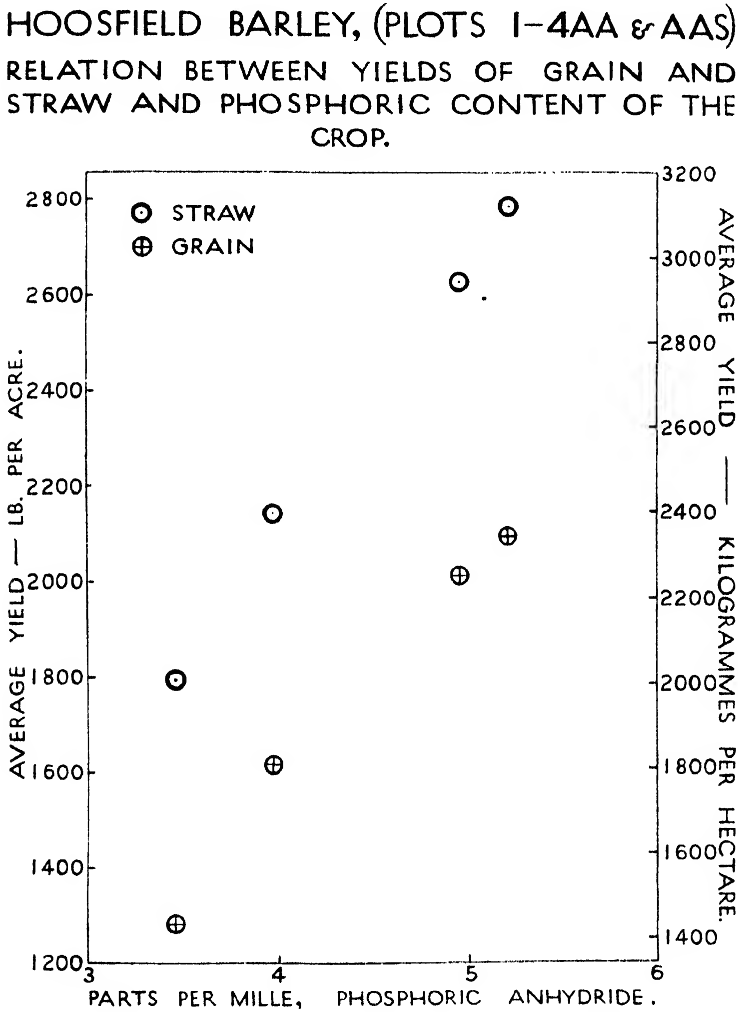
Table VI. *Phosphoric anhydride per mille dry matter.*

	Plot 1	Plot 2	Plot 3	Plot 4	Standard error
		1868–73.			
Series AA	3·67	4·69	3·60	4·82	—
Series AAS	4·09	4·88	4·08	5·21	
		1903–4.			
Series AA	3·46	5·36	3·08	5·19	—
Series AAS	4·21	5·54	3·92	5·80	
		1906–11.			
Series AA	3·36	5·10	3·34	4·97	—
Series AAS	3·85	5·25	3·80	5·20	
		14 years.			
Series AA	3·51	4·96	3·42	4·94	0·049
Series AAS	4·00	5·13	3·94	5·29	

The table (Table VI) shows at all periods separately that the phosphatic content of the dry matter of the crop is increased in all four plots. It is to be noticed that the two years for which analyses are given by Hall and Morison are not the least striking in this respect. Once the results are expressed in this form, however, there can be little doubt that the addition of silicate has had at all periods, and on all the plots, the effect of making more phosphoric acid available to the crop, and not merely of stimulating growth with the secondary effect that more phosphoric acid is absorbed.

6. Crop Increase in Relation of Abundance of Phosphate.

The conclusion of the last section raises the further question as to whether the crop increase associated with the addition of silicate is not



wholly accounted for by the increased abundance of available phosphate which such addition produces. We have no direct evidence of the amount of crop increase which would have been induced by smaller

additions of superphosphate than that actually employed. It is, however, reasonable to suppose that if we had the results of applying phosphate in a number of separate increments, the relation between average yield either in grain or straw and the phosphatic content of the plant would be represented by a smooth curve.

For this purpose plots 1 and 3 which differ only in the potassic fertiliser, and plots 2 and 4, may be thrown together and we obtain the results of Table VII.

Table VII.

	Phosphoric anhydride per mille dry weight	Grain. Mean yield 59 years bus. per acre	Straw. Mean yield 59 years lb. per acre
Plots 1 and 3	3·462	24·66	1794
Plots 1 S and 3 S	3·971	31·06	2144
Plots 2 and 4	4·951	38·68	2625
Plots 2 S and 4 S	5·210	40·30	2782

The values are represented graphically in the figure.

It will be seen that the values for the grain follow a very regular curve, while those for straw are somewhat less regular. Considering, however, that the standard error of our values for phosphatic content is about 0·035, it appears that neither curve can be regarded as indicating any significant departure from the simple view that the whole of the increased yield both in grain and straw associated with the dressing of sodium silicate is solely ascribable to the increased availability of the phosphatic reserves of the soil. If the results can at all be expressed in terms of stimulus, it is a stimulus to phosphatic intake only, and not to plant growth, that must be postulated.

Summary.

The addition of sodium silicate has been found to increase the yield of barley to a considerable extent, this effect being most marked when no superphosphate is added.

The phosphatic content of the ash is not greatly increased in the grain, and is diminished in one case in the straw; the conclusion from this observation that the silicate does not act by releasing soil phosphates, but as a plant stimulus, overlooks the fact that the addition of silica to the ash naturally reduces the percentage of other constituents, and should be discounted.

The phosphate removed annually in the crop is greatly increased on the plots receiving silicate, even when this removal has continued for many years without replacement.

That additional phosphate is actually made available to the crop on the plots receiving silicate is shown by the increase in the proportion of phosphate in the dry weight of the crop, which appears on all the plots, and at all periods.

This increase is quantitatively sufficient to account for the increased yield in grain and straw, without postulating the aid of any stimulus to plant growth.

REFERENCE.

(1) HALL, A. D. and MORISON, C. G. T. On the Function of Silica in the Nutrition of Cereals. *Proc. Roy. Soc.* (1906), B, **77**, 455-477. (Rothamsted Memoirs, vol. VIII.)

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A STUDY OF THE METABOLISM OF TWO BREEDS OF PIG

(WITH SOME REMARKS ON A THIRD).

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(*From the Institute of Animal Nutrition, School of Agriculture, Cambridge.*)

(With Twelve Text-figures.)

CONTENTS.

Introductory.

The animals used in the investigations to be described were (*a*) a pedigree Berkshire hog obtained from Mr H. R. Beeton of Checkendon, Reading; farrowed April 2, 1925, and weaned and castrated before being brought to the laboratory on June 5, 1925—this being appreciably earlier than Mr Beeton was in the habit of weaning—and (*b*) a pedigree Middle White hog supplied by Messrs Chivers and Son of Histon, Cambridge; farrowed August 27, 1925, castrated October 21, 1925, and weaned the day following.

The meal supplied throughout to both pigs was composed as follows:

Barley meal	65 %
Sharps	25 %
Fish meal	10 %

The ration for the day was made up with water and fed, half in the morning at about 9.0 a.m. and half in the evening at approximately 4.0 p.m. The daily ration of meal (not dry matter) is shown in Table I for the different live weights of the pigs; but the Middle White was kept on 3 lb. per day right up to about 200 lb. weight owing to a misunder-

standing when the animals were moved from a pen in the grounds of the School of Agriculture to the University Farm.

Table I. *Daily rations supplied to experimental pigs.*

Weight of pig lb.	Meal lb. oz.	Weight of pig lb.	Meal lb. oz.
15–25	1 0	126–175	3 12
26–40	1 6	176–225	4 8
41–63	1 14	226–275	5 4
64–88	2 6	276 and over	6 0
89–125	3 0		

Note to Table I.

On June 6, 1927, the ration of the Middle White pig which had reached a live weight of something over 350 lb. was reduced to 4 lb. per day on the advice of Mr H. R. Davidson. The pigs received an occasional cabbage or other green food in addition to the above.

The metabolism measurements in both cases have been made with the instrument described by the writer in a previous publication (1), and in the case of the Middle White at the heavier weights in a new and larger instrument on similar lines erected since the date of the above.

The growth curves of both pigs were normal, though the rate of growth was naturally less than is usual owing to the exceptional treatment they received.

EXPERIMENTAL RESULTS.

The animals were placed in the calorimeter at about 10.0 a.m. and fed in the instrument at 4.0 p.m. with the last half of the day's ration. The scheme of observing has not been changed lately. Test observations are made at 6.0 p.m. as many alterations, in electric pressure, etc. take place about 5.30 p.m., then from about 10.0 p.m. the observer awaits suitable opportunities for making observations of the metabolism while the animal is asleep; thus an observation is obtained in Calories per minute at a time when it is known to have been constant at that figure for some time. Observations are made for periods up to five or six nights according to the age of the pig, and the best observation of each night is chosen in the manner indicated by Capstick and Wood (2).

To emphasise the fact that the observations are made in Calories per minute *at a particular moment* they are entered thus in Tables II *a* and II *b*, which together form the chief table of *uncontaminated* experimental data, but later in the paper they will often be found converted into Calories per hour, as this is the more usual way of returning them when, in other laboratories, they are determined as integrals over an hourly or half-hourly period.

Table II *a*. *Experimental data secured with the Berkshire pig.*

(Small calorimeter)

Date	No. of hours fasting	Age of pig in days	Length in inches	Weight in lb.	Body temperature °F.	Observed metabolism Cals. per min.	Mean temperature of environmental air °C.	Metabolism corrected to 16·5° C. Cals. per min.	Observed fasting katabolism Cals. per min.	At °C.	Fasting katabolism from corrected observations Cals. per min.
8. vi. 25	—	67	—	35	—	—	—	—	—	—	—
9. vi. 25	11	68	—	—	—	0·750	17·3	0·775	—	—	—
11. vi. 25	60	70	—	—	—	0·724	18·1	0·760	—	—	—
12. vi. 25	82½	**71**	—	**34**	—	**0·622**	18·4	**0·660**	**0·620**	18·4	**0·660**
22. vi. 25	—	81	—	42	102·3	—	—	—	—	—	—
23. vi. 25	12	82	—	—	—	0·897	17·8	0·937	—	—	—
24. vi. 25	38	83	—	—	—	0·679	17·4	0·704	—	—	—
25. vi. 25	58	84	—	—	—	0·626	17·2	0·644	—	—	—
26. vi. 25	82	**85**	—	**36**	99·2	**0·636**	17·0	**0·648**	**0·630**	17·1	**0·645**
14. vii. 25	—	103	—	53	102·0	—	—	—	—	—	—
15. vii. 25	12	104	—	—	—	0·930	18·7	1·000	—	—	—
16. vii. 25	36	105	—	—	—	0·716	18·8	0·766	—	—	—
17. vii. 25	60	**106**	—	**47**	97·4	**0·695**	19·3	**0·755**	**0·695**	19·3	**0·755**
3. viii. 25	4	123	—	68	103·3	1·397	18·0	1·472	—	—	—
4. viii. 25	10½	124	—	—	—	1·150	18·0	1·212	—	—	—
5. viii. 25	34½	125	—	—	—	0·840	17·8	0·877	—	—	—
6. viii. 25	58	126	—	—	—	0·797	18·3	0·845	—	—	—
7. viii. 25	83	127	—	—	—	0·740	18·4	0·785	—	—	—
8. viii. 25	108	**128**	—	**60**	97·7	**0·731**	18·3	**0·774**	**0·735**	18·3	**0·780**
24. viii. 25	6½	144	—	78	102·7	1·170	19·4	1·275	—	—	—
26. viii. 25	36	146	—	—	—	0·803	18·7	0·851	—	—	—
27. viii. 25	59	147	—	—	—	0·729	18·7	0·772	—	—	—
28. viii. 25	84	148	—	—	—	0·680	18·4	0·722	—	—	—
29. viii. 25	109	**149**	—	**71**	97·6*	**0·670**	18·6	**0·720**	**0·675**	18·5	**0·720**
12. x. 25	—	193	—	113	103·0	—	—	—	—	—	—
13. x. 25	11	194	—	—	—	1·271	17·6	1·319	—	—	—
14. x. 25	35	195	—	—	—	0·977	17·4	1·013	—	—	—
15. x. 25	61	196	—	—	—	0·883	17·0	0·899	—	—	—
16. x. 25	84	197	—	—	—	0·847	17·1	0·865	—	—	—
17. x. 25	108	**198**	—	**101**	99·6	**0·797**	16·7	**0·801**	**0·795**	16·7	**0·810**
2. xi. 25	—	214	—	124	101·8	—	—	—	—	—	—
3. xi. 25	11½	215	—	—	—	1·923	18·5	2·058	—	—	—
4. xi. 25	34½	216	—	—	—	1·222	17·9	1·286	—	—	—
5. xi. 25	58½	217	—	—	—	1·046	17·5	1·086	—	—	—
6. xi. 25	83½	218	—	—	—	0·938	17·3	0·968	—	—	—
7. xi. 25	107	219	—	—	—	0·867	17·1	0·886	—	—	—
8. xi. 25	133	**220**	—	**111**	†	**0·842**	16·9	**0·855**	**0·855**	17·0	**0·870**
30. xi. 25	—	242	—	147	103·7	—	—	—	—	—	—
1. xii. 25	10½	243	—	—	—	2·287	18·1	2·422	—	—	—
2. xii. 25	34½	244	—	—	—	1·311	17·4	1·357	—	—	—
3. xii. 25	58½	245	—	—	—	1·280	16·7	1·288	—	—	—
4. xii. 25	82½	246	—	—	—	1·117	16·9	1·135	—	—	—
5. xii. 25	106½	**247**	—	**137**	99·8	**0·979**	16·8	**0·985**	**1·020**	16·8	**1·025**
25. i. 26	—	298	—	186	103·1	—	—	—	—	—	—
26. i. 26	12	299	—	—	—	2·030	18·1	2·150	—	—	—
27. i. 26	34	300	—	—	—	1·358	18·2	1·438	—	—	—
28. i. 26	57½	301	—	—	—	1·271	18·5	1·359	—	—	—
29. i. 26	83	302	—	—	—	1·164	18·6	1·246	—	—	—
30. i. 26	107	**303**	—	**178**	99·5	**1·120**	18·6	**1·204**	**1·140**	18·6	**1·220**

* Rose rapidly after feeding reaching normal < 10 hours after feeding.

† Thermometer broke.

Table II *b*. *Experimental data secured with the Middle White pig.*

(*Small calorimeter*)

Date	No. of hours fasting	Age of pig in days	Length in inches	Weight in lb.	Body temperature °F.	Observed metabolism Cals. per min.	Mean temperature of environmental air °C.	Metabolism corrected to 16·5° C. Cals. per min.	Observed fasting katabolism Cals. per min.	At °C.	Fasting katabolism from corrected observations Cals. per min.
26. x. 25	6½	60	—	19	101·6	0·492	17·0	0·492	—	—	—
28. x. 25	36	62	—	—	—	0·429	17·0	0·429	—	—	—
29. x. 25	59½	63	—	16	101·6	0·372	17·2	0·372	0·370	17·2	0·370
16. xi. 25	—	81	—	25	103·5	—	—	—	—	—	—
17. xi. 25	13½	82	—	—	—	0·690	17·4	0·690	—	—	—
18. xi. 25	34	83	—	—	—	0·508	17·2	0·508	—	—	—
19. xi. 25	59	84	—	—	—	0·453	17·5	0·453	—	—	—
19. xi. 25	60½	84	—	23	101·4	0·458	17·5	0·458	0·455	17·5	0·455
7. xii. 25	—	102	—	39	103·9	—	—	—	—	—	—
8. xii. 25	12½	103	—	—	—	0·989	16·9	0·989	—	—	—
9. xii. 25	35	104	—	—	—	0·809	17·4	0·809	—	—	—
10. xii. 25	59	105	—	—	—	0·652	18·4	0·652	—	—	—
11. xii. 25	80½	106	—	33	101·8	0·603	18·7	0·603	0·605	18·7	0·605
5. i. 26	7	132	—	48	102·4	0·926	16·5	0·926	—	—	—
7. i. 26	11	133	—	—	—	0·866	16·8	0·866	—	—	—
8. i. 26	35	134	—	—	—	0·745	17·1	0·745	—	—	—
9. i. 26	59	135	—	—	—	0·665	17·4	0·665	—	—	—
10. i. 26	82	136	—	42	100·2	0·661	17·9	0·661	0·660	17·9	0·660
1. ii. 26	—	158	—	62	103·9	—	—	—	—	—	—
3. ii. 26	36	160	—	—	—	0·808	16·9	0·808	—	—	—
4. ii. 26	60	161	—	—	—	0·778	16·5	0·778	—	—	—
5. ii. 26	82½	162	—	56	100·4	0·714	16·5	0·714	0·715	16·5	0·715
1. iii. 26	—	186	—	81	102·3	—	—	—	—	—	—
2. iii. 26	11½	187	—	—	—	1·329	16·6	1·329	—	—	—
3. iii. 26	35	188	—	—	—	1·005	16·3	1·005	—	—	—
4. iii. 26	61½	189	—	—	—	0·900	16·4	0·900	—	—	—
5. iii. 26	83	190	—	72	99·8	0·928	15·8	0·924	0·915	16·1	0·915
29. iii. 26	—	214	—	97	103·5	—	—	—	—	—	—
30. iii. 26	11	215	—	—	—	1·556	16·1	1·556	—	—	—
31. iii. 26	33½	216	—	—	—	1·154	16·2	1·154	—	—	—
1. iv. 26	58	217	—	—	—	1·084	16·4	1·084	—	—	—
2. iv. 26	82	218	—	—	—	1·032	16·4	1·032	—	—	—
2. iv. 26	83½	218	—	88	99·0	1·013	16·4	1·013	1·020	16·4	1·020
26. iv. 26	—	242	—	113	104·0	—	—	—	—	—	—
27. iv. 26	11	243	—	—	—	1·455	16·8	1·455	—	—	—
28. iv. 26	34	244	—	—	—	1·172	17·0	1·172	—	—	—
29. iv. 26	59	245	—	—	—	1·118	17·1	1·118	—	—	—
30. iv. 26	83	246	—	—	—	1·131	17·1	1·131	—	—	—
1. v. 26	108	247	—	102	99·2	0·995*	17·3	0·995	1·125	17·1	1·125

(*Large calorimeter*)

Date	No. of hours fasting	Age of pig in days	Length in inches	Weight in lb.	Body temperature °F.	Observed metabolism Cals. per min.	Mean temperature of environmental air °C.	Metabolism corrected to 16·5° C. Cals. per min.	Observed fasting katabolism Cals. per min.	At °C.	Fasting katabolism from corrected observations Cals. per min.
8. viii. 26	—	346	—	209	103·5	Experiment rejected on account of illness of pig					
14. viii. 26	108	352	32½	196	101·3						
6. ix. 26	6	375	—	218	103·2	2·343	18·6	2·343	—	—	—
7. ix. 26	32	376	—	—	—	1·533	18·3	1·533	—	—	—
9. ix. 26	59	378	—	—	—	1·509	18·3	1·509	—	—	—
10. ix. 26	83	379	—	—	—	1·537	18·3	1·537	—	—	—
11. ix. 26	109	380	32	204	100·4	1·519	18·4	1·519	1·520	18·4	1·520

* There is nothing to account for this very low value.

Table II *b* (*contd.*)

(*Large calorimeter*)

Date	No of hours fasting	Age of pig in days	Length in inches	Weight in lb.	Body temperature °F.	Observed metabolism Cals. per min.	Mean temperature of environmental air °C.	Metabolism corrected to 16·5° C. Cals. per min.	Observed fasting katabolism Cals. per min.	At °C.	Fasting katabolism from corrected observations Cals. per min.
15. xi. 26	—	445	—	266	103·6	—	—	—	—	—	—
16. xi. 26	12	446	—	—	—	2·311	13·4	2·018	—	—	—
17. xi. 26	37	447	—	—	—	1·909	13·4	1·667	—	—	—
18. xi. 26	60½	448	—	—	—	1·865	13·4	1·628	—	—	—
19. xi. 26	82	449	—	—	—	2·016*	13·5	1·775	—	—	—
20. xi. 26	109	**450**	34	**252**	99·9	**1·906**	13·3	**1·655**	**1·885**	13·4	**1·640**
13. xii. 26	—	473	—	281	103·2	—	—	—	—	—	—
14. xii. 26	12	474	—	—	—	2·469	15·5	2·444	—	—	—
15. xii. 26	34	475	—	—	—	1·814	15·2	1·774	—	—	—
16. xii. 26	58	476	—	—	—	1·803	15·0	1·746	—	—	—
17. xii. 26	82½	477	—	—	—	1·736	15·0	1·682	—	—	—
18. xii. 26	108½	**478**	36	**269**	101·5	**1·743**	15·1	**1·700**	**1·740**	15·0	**1·690**
7. ii. 27	—	529	—	321	104·0	—	—	—	—	—	—
8. ii. 27	14	530	—	—	—	2·585	15·0	2·504	—	—	—
9. ii. 27	33	531	—	—	—	1·964	14·7	1·873	—	—	—
10. ii. 27	60½	532	—	—	—	1·799	14·7	1·712	—	—	—
10. ii. 27	80	532	—	—	—	1·788	14·7	1·701	—	—	—
11. ii. 27	104	**533**	—	—	—	**1·757**	14·7	**1·671**	**1·770**	14·7	**1·685**
12. ii. 27	—	534	36½	**303**	102·0	—	—	—	—	—	—
4. iv. 27	—	585	—	364	104·6	—	—	—	—	—	—
5. iv. 27	10½	586	—	—	—	2·754	15·6	2·730	—	—	—
6. iv. 27	34	587	—	—	—	2·013	15·7	2·003	—	—	—
6. iv. 27	56	587	—	—	—	1·665	15·9	1·665	—	—	—
8. iv. 27	84	589	—	—	—	1·720	15·9	1·720	—	—	—
9. iv. 27	107½	**590**	38	**345**	101·8	**1·688**	15·9	**1·688**	**1·690**	15·9	**1·690**
13. vi. 27	8	655	—	391	101·6	2·270	16·0	2·270	—	—	—
15. vi. 27	36½	657	—	—	—	1·738	16·0	1·738	—	—	—
16. vi. 27	62	658	—	—	—	1·621	16·4	1·621	—	—	—
16. vi. 27	80	658	—	—	—	1·693	16·8	1·693	—	—	—
18. vi. 27	109	**660**	41½	**380**	101·0	**1·742**	17·1	**1·742**	**1·720**	16·9	**1·720**
25. vii. 27	—	697	—	403	101·4	—	—	—	—	—	—
26. vii. 27	9	698	—	—	—	2·087	17·6	2·087	—	—	—
27. vii. 27	36	699	—	—	—	1·759	17·6	1·759	—	—	—
29. vii. 27	83½	701	—	—	—	1·949†	17·8	1·949	—	—	—
30. vii. 27	109	**702**	42	**391**	101·2	1·861†	17·8	1·861	**1·760**	17·6	**1·760**
24. x. 27	—	788	—	448	102·6	—	—	—	—	—	—
25. x. 27	12	789	—	—	—	2·692	16·1	2·692	—	—	—
25. x. 27	31	789	—	—	—	2·007	17·1	2·007	—	—	—
27. x. 27	60	791	—	—	—	1·970	17·1	1·970	—	—	—
28. x. 27	81	792	—	—	—	1·923	17·3	1·923	—	—	—
29. x. 27	111½	**793**	42	**434**	101·1	1·974‡	17·4	1·974	**1·925**	17·3	**1·925**
12. xii. 27	—	837	—	458	102·1	—	—	—	—	—	—
14. xii. 27	37	839	—	—	—	2·068	18·2	2·068	—	—	—
15. xii. 27	61	840	—	—	—	2·149	17·9	2·149	—	—	—
16. xii. 27	85	841	—	—	—	2·230	17·8	2·230	—	—	—
17. xii. 27	109	**842**	41½	**437**	101·3	**1·935**	17·8	**1·935**	**1·935**	17·8	**1·935**

* Pig not asleep.

† Water main burst July 28 and the following night observations were not possible. After this curves were abnormal—it is doubtful if the pig slept at all on the fourth night, while on the last night the metabolism was falling rapidly all the time. The small fall in metabolism from 9 to 36 hours is some justification for drawing the curve as indicated Fig. 1 *b*.

‡ Extrapolated from 109½ hours and probably high.

Notes on Tables II a and II b.

Important dates, figures, etc. are shown throughout in heavy type. The individual experiments will be referred to always by the last date on which metabolism observations were made which is emphasised by heavy type in the tables. The weighings both at the beginning and the end of each experiment were made *before* feeding, the one at the end taking place when the pig was removed from the calorimeter approximately four hours after the last observation are made. Length and body temperature measurements were not made throughout and there are therefore a number of blank spaces under these heads up to April 1926. Lengths, where shown, are measured from the top of the withers to the root of the tail in the manner illustrated by Hogan and Skouby (3).

The mean air temperature inside the calorimeter was taken as the mean of the inlet air and outlet air which may be assumed to give the best estimate of the temperature surrounding the animal for purposes of temperature correction—Capstick and Wood (4) in their work on the effect of temperature on basal metabolism took the mean of the *water* temperatures at entry and exit, but this procedure would not give satisfactory results in the majority of the present cases, as owing to the absence of heating arrangements in the room it was not possible to arrange, as these authors did, that the air should have about the same mean temperature as the water.

In the case of the "Middle White" the actual temperature correction is made from a curve developed later in this article from the observations with the pig in question. For the "Berkshire" the curve deduced for the "Large White" by Capstick and Wood is used, but I make the assumption which they suggest as possible (5) but do not themselves use, namely, that the temperature effect will be proportional to the metabolism. This seems to me the most probable hypothesis since the air temperature must almost certainly operate viâ the skin which is approximately proportional in area to the metabolism during adult life, and even in youth the greater part of the heat eliminated has to pass through the skin. The assumption of anything else would introduce vast difficulties in dealing with metabolisms differing over such a range as those in these experiments when, as is the case, the corrections are not particularly small.

The observed fasting katabolism is taken from the curve of observed metabolisms or, where this is useless as a guide, from the last observation, and is given to the nearest 0·005 Calorie per minute, the temperature

following from the mode of estimation. Similar remarks apply to the fasting katabolism obtained from the corrected observations, but here the temperature is clearly always 16·5°C. I have preferred this figure to that obtained by correcting the observed fasting katabolism for temperature, but the difference is in no case sufficiently great to merit notice.

DESCENT TO FASTING KATABOLISM.

In Figs. 1 *a* and 1 *b* are shown the curves of descent from the normal metabolism to the fasting katabolism plotted from the corrected values of the observations (see col. 9 of Tables II *a* and II *b*). A small amount

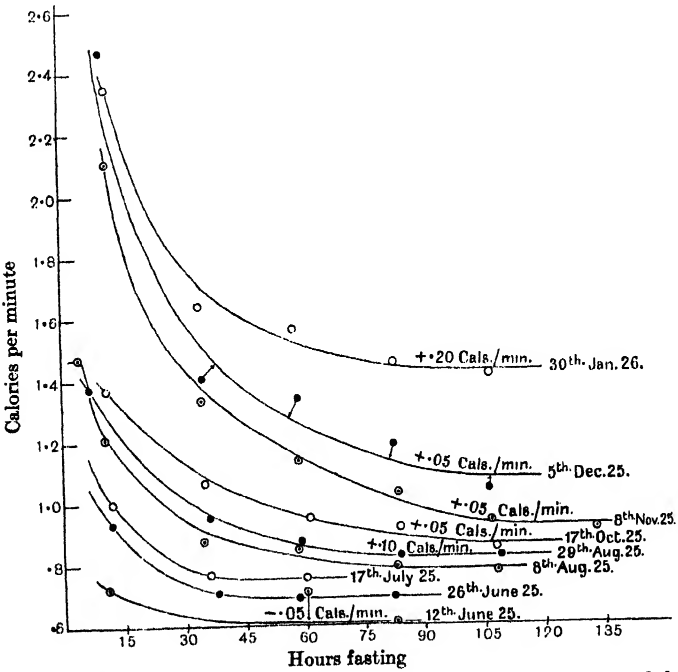


Fig. 1 *a*. Curves of descent to fasting katabolism in experiments with the Berkshire pig.

is occasionally added or subtracted throughout to prevent the curves overlapping, and in these cases indication of the amount of this is made on the curves affected. Two of the curves in Fig. 1 *b* are not completed—these were very poor ones, and in that of July 30, 1927, the points are omitted as they would have got mixed up with those of the other curves; the missing observations will be found duly entered in the proper place in Table II *b*.

These curves are analogous in general shape to those published by the

writer (6) on experiments with a Large White pig. The more rapid fall to the fasting level during youth appears to be a general phenomenon, and that this is only noted in the first three experiments with the

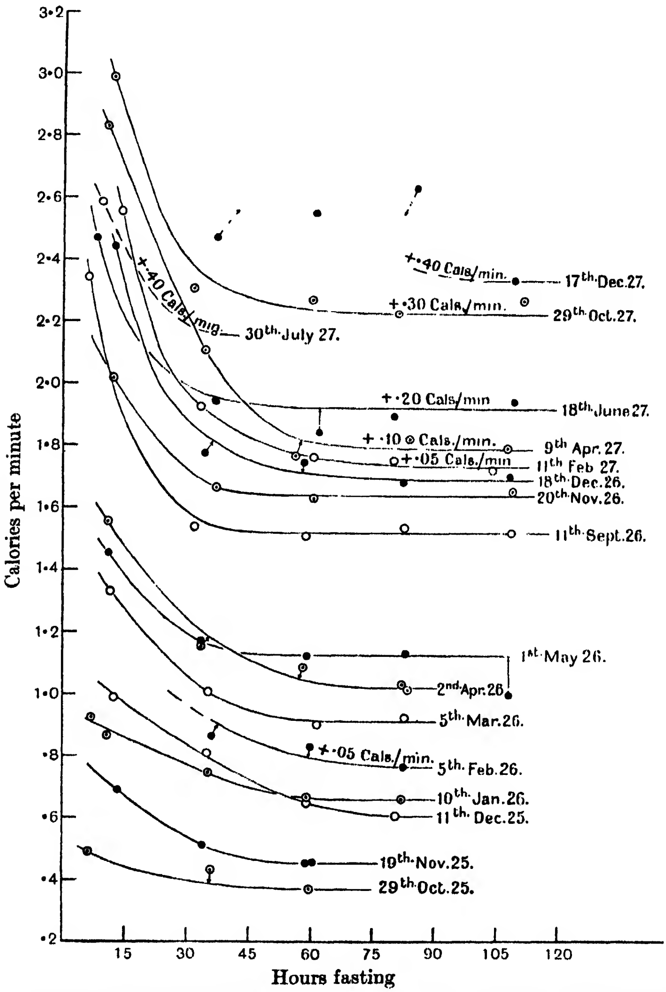


Fig. 1 *b*. Curves of descent to fasting katabolism in experiments with the Middle White pig.

Berkshire pig is quite in accord with what is found in other directions with this animal, which seems to attain to the adult state at a very early age. A maximum of assimilatory activity five or six hours after the ingestion of food also appears to be general.

There seems to be a certain similarity in the mode in which the shape of the curves themselves varies—it is unfortunate that the experiments with the "Middle White" had to be interrupted—but taking those for the Berkshire pig we have a flattened curve on July 17, 1925, followed by two or three normal ones and then a very steep high one on Nov. 8, 1925, followed by a poor curve and a second flattish one on Jan. 30, 1926. A similar sequence may be traced in the curve already published for the Large White (*l.c.*), corresponding dates being Sept. 30, 1922 (first flat curve), Jan. 20, 1923 (steep curve), and April 14, 1923 (second flat curve), and a part of such a sequence may be observed in the curves for the Middle White. It would be unwise to stress the point at the moment, but it seems worthy of passing mention. It was suggested in the paper quoted that these peculiarities might be referable to increases in the ration taking place in steps instead of continuously, but with this further evidence before us the hypothesis seems untenable, *e.g.* when the Middle White pig had been on a ration of 3 lb. of meal from March to August 1926, the sudden increase to 4½ lb. in the latter month and to 5¼ lb. in September in accordance with Table I failed to make the curves of Sept. 11 or Nov. 20 in any way steeper, and in general there is no relation to be traced between the two.

The sharp descent of all the curves during the early hours of fasting leads one to the conclusion that it is at present inadvisable that we should go over to what is the common practice in America and elsewhere, in metabolism experiments upon men, and take the "standard metabolism" or the metabolism in the "post-absorptive" state. In the animals we have worked with it appears that the utmost difficulty would be encountered in getting any satisfactory value at this point, *i.e.* 15 to 24 hours from the beginning of the experiment. Krogh's doubts (7) as to the usefulness of attempting to get a real basal figure on the score that the functional activities of the various organs are responsible for a considerable part of the metabolism, and that only by stopping these, including presumably the heart, could a theoretical basal be obtained, seem countered by the fact that (*a*) a fairly constant figure *can* be obtained after four or five days' fasting, and (*b*) if functional activity of all organs be excluded we can hardly be considered to be dealing with a living animal. It is just possible that observations taken in the morning at about nine or ten o'clock, after say 17 hours' fasting, might be steady for a couple of hours or so, owing to interruption of the fall by superposition of some diurnal rhythm of metabolism with a maximum in the forenoon, of the existence of which we have a certain amount of evidence,

so far unpublished, and which was noted by Johansson (8) in men, and ascribed to the influence of daylight, noise and psychic tone (psychischen Thätigkeitzustand). It is however difficult to see what theoretical value could be ascribed to such a figure. Possibly a similar arresting of the fall may account for the observations of Benedict and Benedict (9) who found that a small breakfast containing in all some 250 Cals. and free from caffeine, protein and ketose sugars did not affect the observations of metabolism if taken an hour before the experiment.

As regards the actual values to which the metabolism fell in the experiments under discussion—the so-called *fasting katabolism*[1]—very considerable differences are apparent between the three pigs concerned, and these will now be discussed in detail.

BODY TEMPERATURE EFFECTS.

That a change in the body temperature of the animal may affect the fasting katabolism observed is admitted, since any cooling of the body must involve a loss of heat by it, thus raising the amount of heat registered by a calorimeter; on the other hand the cooling must slow down the chemical processes of metabolism in which heat is eliminated thus lowering the amount of heat registered.

Of the body temperature readings given in col. 6 of Tables II *a* and II *b* I regard the high ones as due to nervousness of a transitory nature on entering the calorimeters, as on all occasions when a second trial was made after the pig had been inside for some hours results were obtained in the neighbourhood of the normal figure (102·6° F.), and if we take this as our starting point there is then a noticeable tendency for the Berkshire hog's body temperature to fall to a greater extent by about 2° F. than that of the Middle White—the averages are:

Berkshire pig	Final body temperature	98·7° F.
Middle White pig	,, ,,	100·8° F.

The magnitude of the effect of this cooling of the body will depend naturally on how long it takes to cool, but estimating for a "mean pig" of 220 lb. wt. falling 3·6° F. (*i.e.* 100 kg. falling 2° C.) it can hardly amount to more than about 0·03 Cal. per minute over an experiment lasting 100 hours if the fall in body temperature is uniform.

Clearly then we may anticipate that a much greater effect and indeed

[1] I have preferred to use this term where that of basal metabolism has hitherto been employed. It has the considerable merit of conveying the same meaning in all parts of the world, whereas basal metabolism is used by various writers to mean anything from the asting katabolism to the actual metabolism per unit area 12 hours after food.

the only one of the two we need consider will be that due to the slowing of the chemical processes in the body since Du Bois (10) has shown that at any rate under febrile conditions the metabolism of man follows Van 't Hoff's Law, which, being a purely physical relation, makes it reasonable to suppose, until the contrary is proved, that it also holds under *subnormal* conditions where these are maintained for any length of time and also not improbably in other animals than man. It is well known that a low metabolism and a subnormal temperature go hand in hand in the pathological conditions of myxoedema and cretinism.

It seems therefore possible that the low values obtained for the fasting katabolism of the Berkshire pig at weights over 50 lb. may be associated with a slowing down of the chemical processes in its body by this reduction of temperature; but if so, *either* the proportionate fall in metabolism from the normal to the fasting level in the Berkshire pig ought to be about double that exhibited by the Middle White, *or else* the metabolism of the Middle White must fall more per degree reduction of body temperature than that of the Berkshire.

Table III. *Relation of decline in body temperature to fall in metabolism in the two pigs.*

Berkshire pig—temperature fall 3·9° C.				Middle White pig—temperature fall 1·8° C.			
Date of experiment	Metabolism at 12 hrs. Cals. per min.	Fasting katabolism Cals. per min.	% excess of 12 hrs. metabolism over fasting level	Date of experiment	Metabolism at 12 hrs. Cals. per min.	Fasting katabolism Cals. per min.	% excess of 12 hrs. metabolism over fasting level
12. vi. 25	0·765	0·660	15·9	29. x. 25	0·455	0·370	23·0
26. vi. 25	0·937	0·645	45·3	19. xi. 25	0·705	0·455	55·0
17. vii. 25	1·000	0·755	32·5	11. xii. 25	1·000	0·605	65·3
8. viii. 25	1·200	0·780	53·9	10. i. 26	0·875	0·660	32·6
29. viii. 25	1·160	0·720	61·1	5. iii. 26	1·320	0·915	44·3
17. x. 25	1·310	0·810	61·7	2. iv. 26	1·530	1·020	50·0
8. xi. 25	2·040	0·870	134·5	1. v. 26	1·430	1·125	27·1
5. xii. 25	2·200	1·025	114·6	11. ix. 26	1·985	1·520	30·6
30. i. 26	2·150	1·220	76·2	20. xi. 26	2·010	1·640	22·6
				18. xii. 26	2·440	1·690	44·4
				11. ii. 27	2·590	1·685	53·7
				9. iv. 27	2·680	1·690	58·5
				18. vi. 27	2·090	1·720	21·5
				29. x. 27	2·690	1·925	39·8
	Average % excess		**66·2**		Average % excess		**40·6**

To get an idea of what the proportionate fall in metabolism is we may take all possible observations for each pig and find the average percentage by which the metabolism at, say 12 hours from the beginning of the experiment exceeds the fasting katabolism. Then since the average rectal

temperature fall is twice as great in the Berkshire as it is in the Middle White we should expect that if the *whole* of the fall in metabolism were linked with the fall in body temperature the figure thus obtained for the Berkshire pig would be approximately twice as great as the corresponding average for the Middle White.

In Table III the figures are given as suggested above; the metabolism at 12 hours from the commencement being taken from the curves of Figs. 1 *a* and 1 *b* where observations at exactly 12 hours are wanting.

It will be seen that the percentage excess mentioned *is* greater in the Berkshire than in the Middle White pig, and from this one would seem to be justified in concluding that at all events the greater part of the fall in metabolism in these animals is associated with the fall in body temperature—which is the cause and which the effect it is hard to say. A lower heat production might be expected to lower the body temperature, on the other hand, if the body temperature were otherwise lowered, as for example by more rapid radiation of heat from the surface of a black pig like the Berkshire than from that of a white one like the Middle White, heat production being assumed equal in the two, it would seem to be an unavoidable consequence that the metabolism should follow suit. Of these alternatives one would naturally choose the former were it not for Benedict and Ritzman's results (11) from which it appears that, at any rate in steers, a fall in metabolism, even if considerable, is not necessarily associated with a fall in body temperature. We are driven then to at least the serious consideration of the second possibility, and one would be interested to see how other work on the influence of skin colour on metabolism bears upon this, but the only memoirs I have been able to find are those of de Almeida (12), who found the negro metabolism averaged about 8 per cent. higher than that of whites under similar conditions in Brazil. On the grounds that this figure was appreciably less than the mean error (écart moyen) of his observations, de Almeida concluded that no inference could be drawn from his results in the sense that the metabolism of negroes was on a higher level than that of whites acclimatised to the tropics. He quotes however $\sqrt{\frac{\Sigma(x^2)}{n-1}}$ as the formula for computing the mean error, and I have verified that this was the one actually employed, whence it is clear that he is dealing with the mean error of a *single observation* or standard deviation. The formula which should have been used is that for the mean error of the arithmetic mean, namely $\sqrt{\frac{\Sigma(x^2)}{n(n-1)}}$. Applying this to de Almeida's results we find that

the mean error works out at something less than $3\frac{1}{2}$ per cent., thus the 8 per cent. difference in the means of the observed figures becomes of rather greater significance.

Environmental Temperature Effects.

It was stated on p. 145 that a special temperature correction curve had been worked out for the Middle White pig. Some difficulty was experienced in correcting the results obtained from the curve of Capstick and Wood (4), and eventually a method of successive approximation was devised which led to better results with this pig and incidentally to the discovery of its exceedingly low critical temperature.

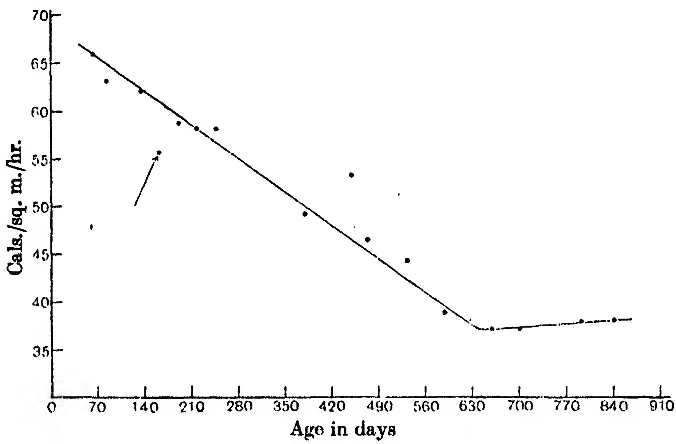


Fig. 2. Uncorrected values of fasting katabolism per unit surface plotted against age (Middle White pig).

Taking the uncorrected values of the fasting katabolism, reducing these to Calories per hour per square metre surface area and plotting them against the age of the pig in days we get the curve of Fig. 2.

By making the approximate assumption that the uncorrected curve of Fig. 2 does actually represent the effect of *age* correctly[1], then supposing that the metabolism per unit area eventually becomes constant at 37 Calories per square metre per hour, we can argue backwards to the *approximate* temperature correction curve. Thus, since we have assumed (p. 145) that the correction is proportional to the metabolism we must first of all reduce all observations of metabolism per unit area

[1] This differs from the assumption made by Capstick and Wood (*l.c.*) who took only points at *nearly* the same temperature and then corrected these to one temperature by a law *presumed locally linear*, thus obtaining a curve from which the age effect might be deduced.

to a basis of 37 Cals. per sq. metre per hour by dividing by the ratio of the value in Cals. per sq. metre per hour represented by the height of the curve at the time to this basis figure. The amount by which each of the quantities thus found differs from 37 will consist of a part due to the temperature effect, if present, and a part due to experimental error. If therefore we plot these quantities against the temperatures at which the observations to which they refer were made we shall obtain an approximate temperature correction curve. Moreover we can now, if we wish, use this to correct the curve of Fig. 2 obtaining thereby a curve of fasting katabolism per unit area plotted against age which is approximately corrected for environmental temperature. This in its turn may be used as that of Fig. 2 was used before and become the starting point for a more accurate determination, and so on until it is clear that only experimental errors remain.

By way of illustration the first approximation, in this case the only one needed, is shown in Table IV, and the resulting deviations are plotted against observation temperatures in Fig. 3. The observation indicated with an arrow in Fig. 2 was abnormal and is omitted.

Table IV. *Illustrating the application of the method of successive approximation for finding the effect of environmental temperature on the fasting katabolism.*

Date of experiment	Uncorrected fasting katabolism Cals./m.^{2}/hr.	Height of curve = H	37/H	Reduced observation	Deviation from 37	Temperature of experiment ° C.
29. x. 25	66·0	66·0	—	37·0	Nil	17·2
19. xi. 25	63·2	65·0	0·569	36·0	−1·0	17·5
11. xii. 25	66·2	63·9	0·579	38·3	+1·3	18·7
10. i. 26	62·1	62·3	0·594	36·9	−0·1	17·9
5. iii. 26	58·8	59·6	0·621	36·5	−0·5	16·1
2. iv. 26	58·2	58·2	—	37·0	Nil	16·4
1. v. 26	58·2	56·7	0·653	38·0	+1·0	17·1
11. ix. 26	49·2	50·0	0·740	36·4	−0·6	18·4
20. xi. 26	53·3	46·5	0·796	42·4	+5·4	13·4
18. xii. 26	46·5	45·1	0·820	38·2	+1·2	15·0
11. ii. 27	44·3	42·3	0·875	38·8	+1·8	14·7
9. iv. 27	38·8	39·5	0·937	36·3	−0·7	15·9
18. vi. 27	37·1	37·1	—	37·0	Nil	16·9
30. vii. 27	37·1	37·3	0·992	36·8	−0·2	17·6
29. x. 27	37·9	37·8	0·979	37·1	+0·1	17·3
17. xii. 27	38·0	38·0	—	37·0	Nil	17·8

From the lie of the points in Fig. 3 it seems clear that the temperature correction at anything above about 16° C. is nil, *i.e.* as if this were the critical temperature for this pig. The result is remarkable considering that in a Large White it is near to 21° C. according to Capstick and Wood (13), while Tangl (14) using Yorkshires and Mangalicas found about

the same value. The points on the curve are however sufficiently numerous and lie distributed about it with an evenness which renders the acceptance of a lower value for this particular pig unavoidable.

Below about 16° C. the curve rises sharply. The scarcity of points here is regrettable, but in the circumstances there is nothing to be done but to run a curve through them as they stand[1]. The corrected curve is shown in Fig. 4.

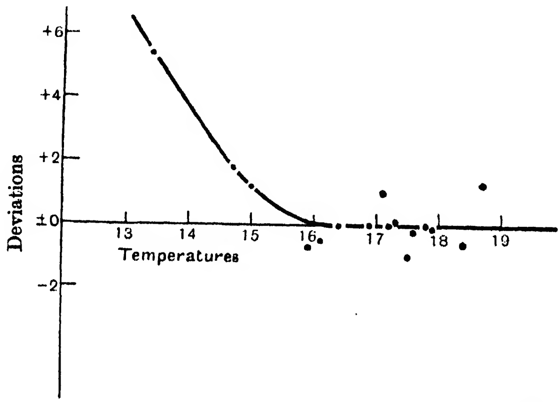


Fig. 3. Showing deviations from 37 plotted against temperatures of experiment.

It has seemed worth while recording this method of approximation in some detail as it appears capable of rendering considerable service as a time saver in the laboratory in the future, since by arranging the work so that each animal is experimented with during its growth at many widely distributed temperatures, the temperature correction and critical temperature can be deduced from experiments which also serve for the determination of the age variation of fasting katabolism at fixed temperatures, and where experiments often extend over years this point is not unimportant. The method is not applicable if the number of observations is insufficient to determine the general character of the curve in Fig. 2.

[1] This procedure puts the observations to which these three points refer actually *upon* the uncorrected curve of fasting katabolism per square metre—but it is well to remark that there is not here anything of the nature of argument in a circle, all it amounts to is that what few points there are which are affected by the temperature correction give deviations so disposed in Fig. 3 that the curve must go exactly through each, if drawn without bias. In effect the same correction curve, as drawn, is used for *all* the observations, some of which do not lie on the line. We assume, in fine, that the experimental error affecting these points is *nil* because there is no indication what it amounts to or in which direction it lies, and of course the points do not lie *exactly on* the final curve, Fig. 4.

The slight upward trend of the curve after it has become to all intents and purposes horizontal in Fig. 4 is, I am convinced, really present. In Fig. 5 which gives the corresponding curve for the Berkshire pig it is

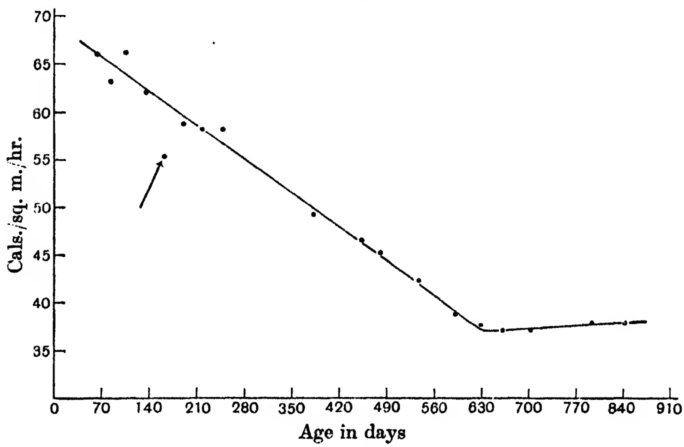


Fig. 4. Corrected values of fasting katabolism per unit area plotted against age (Middle White pig).

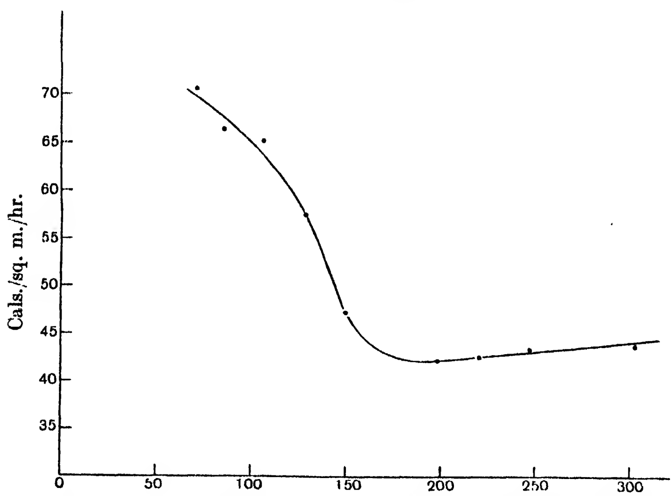


Fig. 5. Corrected values of fasting katabolism per unit area plotted against age (Berkshire pig).

also noticeable and there is at least an indication of it in the case of the Large White previously cited. I am unable to offer any satisfactory reason for it—it may perhaps represent the error due to estimating surface by the two-thirds power of the weight; but the slope is in the

same direction and rather steeper if Hogan and Skouby's (15) length-weight formula for swine is used. It may be noted that to make it horizontal it would be necessary to assume the surface area to vary as a power of the weight greater than two-thirds, not less as in most variants on the simple two-thirds power relation, indeed the relation surface $\propto$ (weight)$^{\cdot 72}$ of Dreyer, Ray and Walker (16) would make this part of the curve practically horizontal. This slight slope makes no practical difference from the point of view of rationing.

Fasting Katabolism per unit Surface Area.

The age at which approximate constancy of fasting katabolism per unit area is reached varies greatly in the three kinds of pig we have under consideration; the figures are:

Berkshire pig	175 days
Large White pig	370 ,,
Middle White pig	630 ,,

In the actual value to which the metabolism sinks there are distinct but smaller differences, the Large White and Berkshire being together in this respect at 42–43 Calories per square metre per hour, while the Middle White becomes steady at the very low figure of 37 Calories per square metre per hour.

As regards the general shape of the curve, the Large White and Berkshire both have a point of inflexion in the middle of the descending curve, but in the Middle White this is conspicuously absent, and the only one in which a distinct "hump" or maximum is reached in early life is that of the Large White. This is a matter of considerable theoretical interest, and the question arose as to whether the maximum that had been found in the Large White pig was real or due to the first observation of that series being inexplicably low. To settle the point the metabolism of another Large White was measured when very young with the result that *no maximum was found*; a third however showed *a distinct maximum.* No other pigs have shown this, but it has been obtained with a Dexter calf. All these results are as yet unpublished. It would appear therefore as if this maximum in the metabolism per unit area in fasting was a somewhat elusive thing. The writer was however struck by the fact that although no maximum is shown by the curves of Figs. 4 or 5, yet if the fasting katabolisms are plotted against age or weight the curves are of similar character for all the pigs, and he was led from this to the consideration that if the rise is due to extra energy required for growth as was

surmised in the work on the Large White (17) then it must begin sometime, rise to a maximum and eventually end as it started. Thus it appeared that there might well be something to be learned from plotting the actual excess metabolism (over that demanded by the surface law) against age. In this way the curves shown in Fig. 6 have been deduced for each of the three pigs.

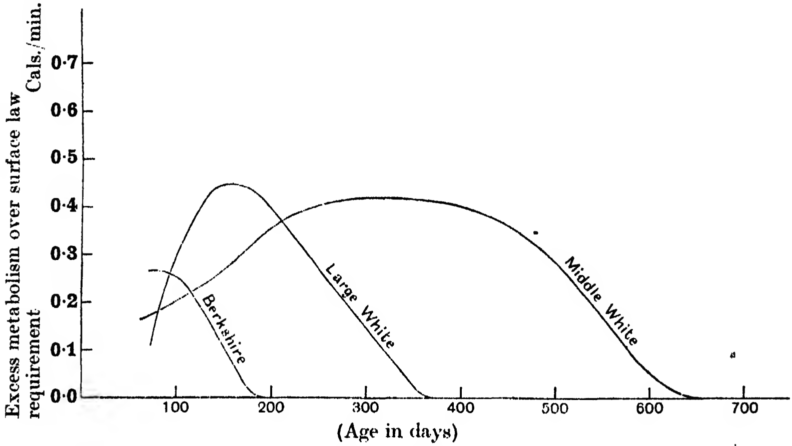


Fig. 6. Showing excess metabolism over that demanded by the surface law at different ages (fasting values).

In this case there is a distinct rise to a maximum in the Middle White pig also and an indication that in the Berkshire the experiments begin at the maximum point.

It will be observed that the Large White makes all this demand for extra energy above the requirements of the surface law *after* birth (0 days). The Middle White may well require a little of it and the Berkshire appreciably more of it *before* birth; so that on this showing we should expect that the Large White sow would be in a better position to produce large litters than either of the others as the drain on her resources for the energy requirements of the young would be less per foetus, *caeteris paribus*, than in the other two breeds. Similarly one would expect the Middle White to excel the Berkshire in this respect. That this is what is found in practice with these three breeds of pig is well known.

To revert to the question of the maxima, I am disposed to think their existence either before or after birth to be a matter not only of physiological probability but of mathematical necessity in both cases, given the admitted fact that there is a greater fasting katabolism per unit area in youth.

Take, for example, the curve of Fig. 4 in which, as we have seen, the maximum is not found in the range covered by the experiments. This shares with all other similar curves the property that there is a definite fall in the fasting katabolism per unit area with advancing maturity. Now if we imagine the possible course of this curve as it passes further to the *left*, it is clear that from a mathematical point of view it may do one of three things:

1. It may bend downwards again, and in this case there clearly *is* a maximum.

2. It may turn over and become horizontal, that is to say, the fasting katabolism per unit area may become established at a higher but constant level.

3. It may continue to ascend, that is the fasting katabolism per unit area may continue to increase.

In case 2, as the volume of the animal, foetus or embryo decreases with diminishing age we have within limits of accuracy sufficient for the present purpose the following relation holding

$$\frac{\text{Surface}}{\text{Volume}} \propto \frac{1}{\text{Linear dimensions}},$$

any small deviation from this depending on shape changes. Now the energy metabolised per minute per unit *volume* of the animal will continually increase as the linear dimensions decrease, since, being by hypothesis proportional to the surface, it varies as the reciprocal of these; this means that for every cubic millimetre of an embryo one millimetre long the heat generated per unit time will have to be 1000 times as great as that developed per cubic millimetre by an animal one metre in length, a quite unthinkable proposition and contrary to all experience. In fermenting yeast according to Brown (18) the heat production is, weight for weight, 70 times that of a man at rest, but as he points out this is only produced under conditions differing radically from those in the natural habitat of yeast on the outer skin of fruits, or even when naturally multiplying where an abrasion has allowed the tissues of these to be invaded.

The same argument holds with augmented force in the third case suggested.

We may therefore safely conclude that either in late uterine life, at birth or shortly after birth there is a time in the life of every animal when the fasting katabolism per unit surface is greater than at any other time. Stating the facts thus we do not exclude the possibility of a definite

discontinuity in the curve at birth, though evidence is still to seek in that matter; no discontinuity in the curve was found by Gayda (19) in his calorimetric study of the eggs of the toad from impregnation to the completion of metamorphosis, even at the emergence of the tadpoles from the gelatinous envelope, and a very definite maximum was observed in the metabolism per unit weight and also in that per unit volume occurring as soon as the tadpoles had acquired the ability to swim and feed themselves, but this is complicated by the movements involved.

It is clear that the time of occurrence of the maximum may be very variable even in one breed of animal, and this may explain the experimental results with the Large White pigs, but more evidence is needed.

Taking all the results together it is clear that these effects cannot be ascribed to any seasonal variation in metabolism of large amplitude such as was found by Benedict and Ritzman (20) in control steers on hay maintenance and by Benedict, Miles, Roth and Smith (21) in a group of controls on normal diet in experiments on human beings.

One may probably regard these maxima as the mathematical outcome of the physiological fact that in order that a warm-blooded animal may live, its actual body temperature, and therefore in a less degree its metabolism per unit time per unit volume, must not vary over any considerable range from earliest embryonic life until death; coupled with the fact that the process of tissue building entails in every case some waste of energy as heat. The variation in time of occurrence of the maximum may be due to small divergencies in growth rate and stage of muscular differentiation or development.

The values to which the metabolism finally falls in fasting are interesting. It will be noticed that the exceptionally low final value of 37 Calories per square metre per hour was only attained by that pig whose critical temperature was found to be so extraordinarily low. Now the experimental data were computed to 16·5° C. before the curve of Fig. 5 was drawn, while the experiments with the Large White were made at a uniform temperature of 16·3° C.; if now we correct these values by Capstick and Wood's curve (5) so that they apply to the critical temperature for these pigs we obtain the very close agreement shown below:

	Fasting katabolism at critical temperature Cals./m.^{2}/hr.
Middle White pig	37·0
Large White pig (Capstick and Wood)	37·7
Large White pig (Deighton)	38·4
Berkshire pig	37·5

These figures show a considerable excess over those registered under similar conditions for other animals with similar digestive tracts but provided with insulation of one kind or another, clothes in human beings, coats of hair or fur in animals, *e.g.*

	Cals.
Men (from Du Bois (22), reduced from Sage Normal to two-thirds power law)	34·5
Dog (White Bull terrier, from Anderson and Lusk (23) $k = 10{\cdot}3$)	34·4
Rabbit (from Voit (24), mean of four high temperature observations)	32·4

It appears possible that the surface law of Voit (*l.c.*) may be true with greater accuracy if stated in the form that the fasting katabolism per unit surface of all omnivorous and carnivorous animals at or above their critical temperatures depends only on their natural insulation. That ruminants come out considerably higher than the values here considered is clear from Benedict and Ritzman's results (25) for cattle; nothing of like kind for sheep appears to be available.

Thermic Energy.

If it were possible to get the shape of the curve of descent to fasting katabolism in the early part of the experiment it would seem that the area included between this curve, the energy axis and a line parallel to the time axis drawn at the level of the fasting katabolism should be a measure of the thermic energy and hence of the nett energy of the ration[1]. This is not at present possible, but a near approximation to it may be made by producing the curves of Figs. 1 *a* and 1 *b* backwards until they cut the 6 hour ordinate, then passing horizontally to the energy axis and estimating the area enclosed within the thick lines in Fig. 7.

Now the area mentioned represents 2664 Calories as here drawn, as shown in Fig. 1 *a* it is 2790 Calories, but I have deliberately taken the curve to the left of, instead of through, the highest observation point, lest the area might have been largely increased by a small experimental error in this observation, the curve being so nearly vertical here.

At this time the pig had been for over three weeks on a daily ration of 3 lb. of meal mixture of nett energy content approximately 104 therms per 100 lb. (meal, not dry matter). We may reckon the thermic energy of this at about one-quarter of the amount of the nett energy, since in such pig feeding mixtures the nett energy is sufficiently near to 80 per cent. of the metabolisable.

[1] A certain allowance would have to be made for any meal taken less than 24 hours before the last. Since all observations are made when the pigs are sleeping, and have been so for some time, it cannot contain any muscular energy.

Hence:

	Cals.
Thermic energy due 1½ lb. meal at 4 p.m.	390
+ two-thirds (say) of thermic energy due 1½ lb. meal at 9 a.m.	260
Total	650

It is at once apparent that the energy represented by the area in question in Fig. 7 is vastly greater than anything that can be accounted for by the thermic energy of the food ingested as normally understood.

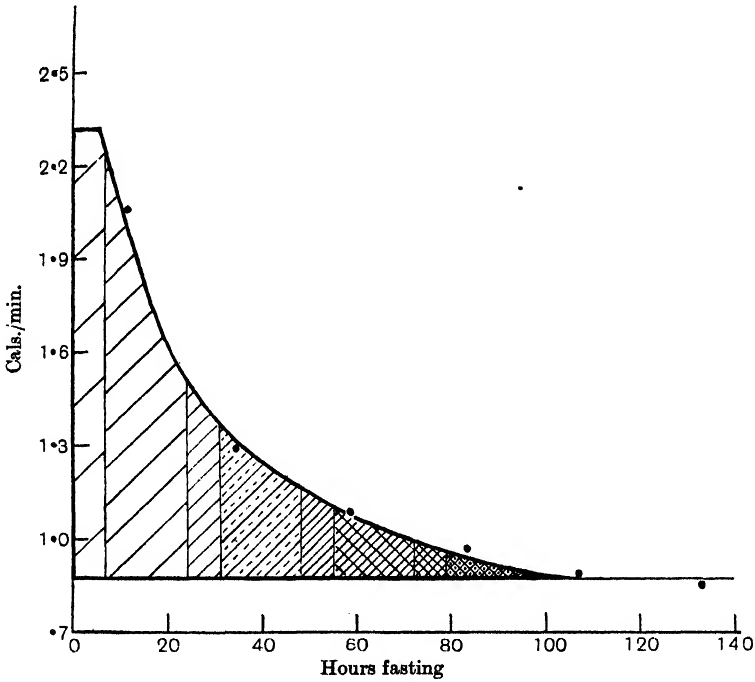


Fig. 7. Showing areas estimated in thermic energy computations (from curve of Berkshire pig, Nov. 8, 1925).

Let us however take the most favourable case in every way in the attempt to "square" this area with the thermic energy. The highest value so far obtained for the increment of heat production with pigs on barley meal is Fingerling's (26) which amounts to 37·6 per cent. of the content of nett energy, the sharps and fish meal will lower this figure to about 36·5 per cent. Moreover, let us assume that not only a fraction of the meal taken at 9.0 a.m. on the day on which the pig entered the calorimeter is to be reckoned in, but that the total thermic energy to be considered is the sum of the remainder effects estimated according to

Fig. 7 of all meals taken by the pig for the previous four days. Thus we assume that the full computed thermic energy is available from the meal taken at the beginning of the fast (4 p.m. Nov. 2, 1925); from that taken at 9.0 a.m. the same day the remainder is estimated as the full computed thermic energy multiplied by the ratio of the shaded area from the 7 hour ordinate onwards (Fig. 7) to the whole area enclosed by the thick lines; from the 4 p.m. meal the previous day it is assumed that a remainder of uneliminated thermic energy is available equal to that computed from the ration multiplied by the ratio of the shaded area from the 24 hours' ordinate onwards to the whole area within the thick lines, and so on. The various shadings are intended to make this easier to follow. Thus we have:

	Cals.
Thermic energy due 1½ lb. meal at 4 p.m. 2. xi. 25 (reckoning 36·5 % of nett energy)	570
Thermic energy due remainder from 1½ lb. meal at 9 a.m. 2. xi. 25	439
,, ,, ,, 4.0 p.m. 1. xi. 25	225
,, ,, ,, 9.0 a.m. 1. xi. 25	175
,, ,, ,, 4.0 p.m. 31. x. 25	93
,, ,, ,, 9.0 a.m. 31. x. 25	62
,, ,, ,, 4.0 p.m. 30. x. 25	24
,, ,, ,, 9.0 a.m. 30. x. 25	15
,, ,, ,, 4.0 p.m. 29. x. 25	2
Total	1605

This figure is still over a thousand Calories short of that estimated from the curve of descent to fasting katabolism. The curve selected is one of the highest. In other cases, such as that with the Middle White ending May 1, 1926, the balance is the other way even on the most conservative estimate of the thermic energy, and in general there appears to be no connection between this area and the computed thermic energy of the ration other than a certain obvious correlation of each with the size of the pig.

The conclusion then appears to be unavoidable, that whatever the shaded area in Fig. 7 may represent, it certainly does *not* represent the thermic energy of the ration.

Energy of Muscular Effort.

It seemed that if some method of estimating the energy expended in the performance of muscular work could be found, even though it could not help in nett energy determination by allowing the form of the first part of the curve to be deduced, yet it might assist in clearing up the problems presented by the results—notably those mentioned in the previous section—by enabling the investigator to exclude it or at least allow for it in suitable manner.

From the curves produced by the recording galvanometers one can see clearly when an animal gets up, as there is a very sharp rise in the curve which then slowly falls again, presumably the areas of these peaks would if integrated and converted to Calories give a total equal to that part of the extra heat generated by movement which has been carried away in the water flow. A quantity of heat suddenly generated inside the calorimeter by electrical means is correctly recorded by the area of the peak formed, but the experimental error in this case counts for considerably more than usual, *e.g.* a *sudden* generation of only 10 Calories in the small calorimeter might be recorded with an error of $60 \times 0{\cdot}02 = 1{\cdot}2$ Calories if the peak took an hour to pass off, since the error is an absolute one of $\pm$ 0·02 Cal./min. and does not change sign with any rapidity. Thus the record of heat due to muscular movement cannot be expected to be accurate unless the period of estimation is long enough for instrumental errors to cancel one another. On the other hand, over the whole time of an experiment, a fairly good estimate of this fraction of the heat due to muscular movement could be made.

In addition to the extra heat given to the water, some is carried out in the air stream and as latent heat in the extra water vapour in this, due to the breathing and insensible perspiration of the animal; both these should strictly speaking be measured, and the question arises, since no automatic record was kept of them, is there any supposition which one can legitimately make regarding the relative magnitude of these three fractions in which the extra heat due to muscular effort is recorded? It is found that over a short space of time, say an hour, the ratio of the total heat recorded to that which is carried out by the water stream alone $= R$ (say) does not alter very greatly, and it might therefore be thought that one could estimate the total extra heat generated by multiplying the excess found in the water stream by R. This is not possible in general, because on occasions when the outlet air has been tested with recording thermometers it has been found that the ratio of total *excess* heat to *excess* heat in the water stream is by no means equal to R. On the other hand, taking a good length of time, say a day, it is found that the mean value, while rather higher under normal circumstances than R, is sufficiently close to it to justify the assumption of equality as an approximation. The value to be taken for R itself must clearly be its mean value over the period of estimation; usually an interpolation by proportional parts to the middle hour of the period will be all that is required.

That the method is invalid when the value of R is too great is obvious

as also is the fact that height of the maxima above the general mean height will vary with the water flow and with the resistance of the thermo-electric circuit.

The computations are cumbrous, but in view of the importance of the matter it seems desirable to demonstrate them in the case of *one* of the curves for which that of Fig. 7 will serve.

The galvanometer charts for the week are shown in Fig. 8.

The greatest difficulty of the whole process is that of deciding where the general mean of resting metabolism has been on any one day. It is plain from Fig. 8 that this mean has lain more or less where the lines below the shaded areas are drawn, but it often happens as on Nov. 3–4, and again on Nov. 4–5, that a considerable difference of opinion may exist on this point. The problem was to devise a few simple rules for drawing these lines which should not be so rigid as to preclude the use of definite knowledge of conditions which were known to have obtained during the experiment, but at the same time might serve to exclude unintentional bias. For this purpose we have the following facts to go upon—we know that the resting metabolism rises for the first 5 or 6 hours of an experiment and then declines, steeply at first and then more gradually in accordance with the curve of descent to fasting level except for a possible diurnal rhythm. We know also that if the ratio R changes by an appreciable amount during the period of 24 hours covered by a chart, it will, if it increases, tend to make the line of minima slope downwards and *vice versa*. Finally one of a series of tangents drawn to the curve of Fig. 7 at time intervals of approximately 24 hours need not intercept a predetermined ordinate situated between its point of tangency and that of the next of the series in the same point as the latter; and neither can such a condition be stipulated as *necessary* for straight lines drawn through the minima of metabolism in any sense that the line on one day must *necessarily* begin at the level above the instrumental zero at which the previous day's line ended, though in most cases this will be very nearly the case. Change of resistance in the thermo-electric circuit will of course also affect this.

In view of these considerations the following rules were drawn up as a guide and the lines so drawn as to satisfy them as far as possible.

Rules for drawing mean lines of minima.

1. The possible diurnal variation to be neglected.

2. The first day of an experiment a rise may be allowed for to 9.0 or 10.0 p.m., where apparently present, with an optional horizontal portion

to 6 p.m. Afterwards a fall to the next morning's change of chart. All these lines to be straight.

3. The second day a falling straight slope in accord with the mean of the night minima. (Day minima affected by lag.)

4. The third and following days a straight line as nearly horizontal as may be in accordance with the night minima.

5. The line of minima each day to follow on from that of the day before as nearly as possible.

6. Portions of galvanometer curve cut off below the mean line of minima to be neglected.

7. Muscular effort estimation to end at the time of observation next after the constant level of fasting katabolism is attained.

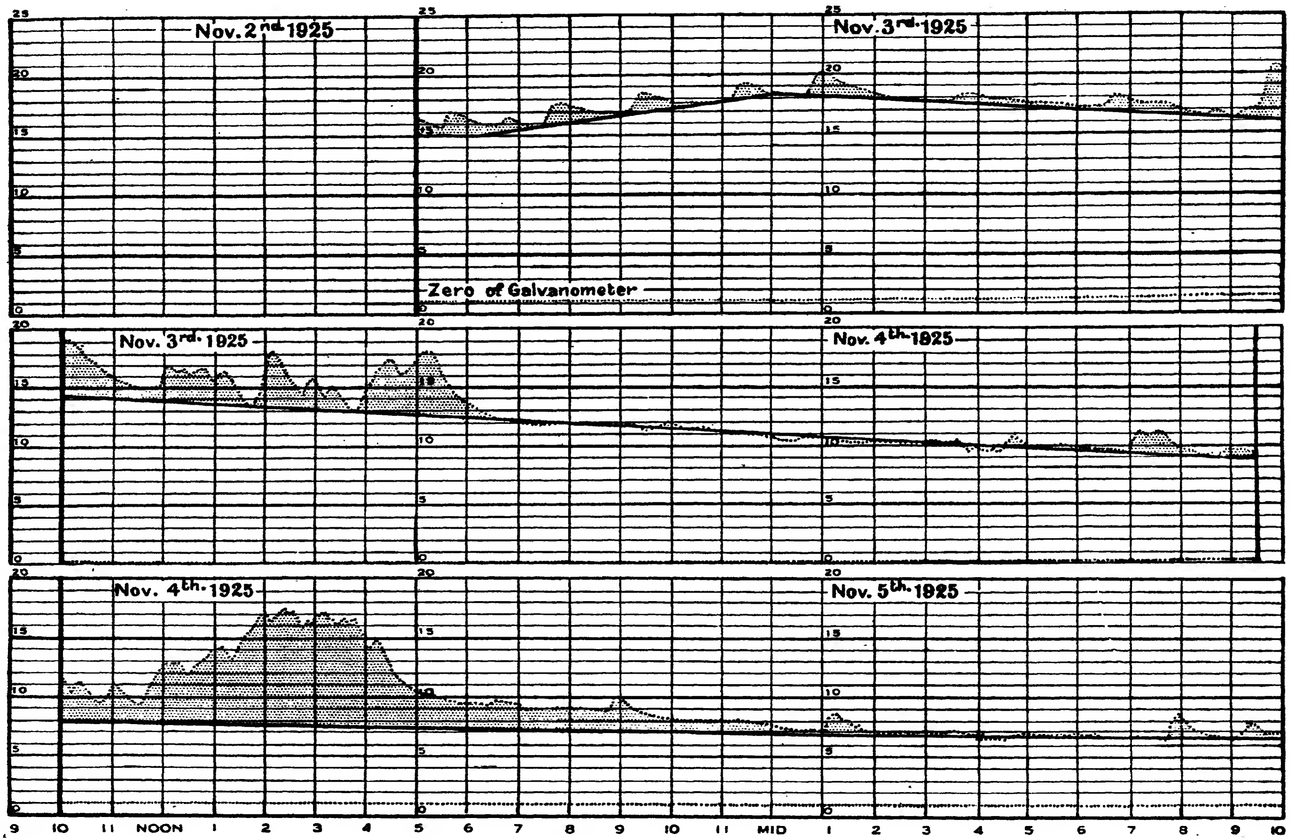
Referring now to the charts in Fig. 8 it will be seen that the latitude is small. The computed areas are shaded. The estimation is not begun until 5 p.m. on Nov. 2 as the rise after 4 p.m. was due to the opening of the door. Between the charts of Nov. 3–4 and Nov. 4–5 half an hour is lost, and another hour and a half when the resistance was changed on the morning of the 5th. The record ends at 5 a.m. on Nov. 8 in accordance with rule 7.

The next step is the estimation with a planimeter of the areas shaded in the charts, Fig. 8, and these results are shown in col. 2 of Table V. It is then necessary to multiply these figures by the average water flow, by the ratio of the actual resistance of the thermo-electric circuit to that of the galvanometer, leads and thermo-couples only (in this case 14·4 ω), and by the ratio R. We thus obtain a figure equal to the area the shaded portion would have had if the water flow had been unity, the extra resistance nil and if all the heat due to muscular movement had been recorded in the water. I have called this figure the "muscular energy proportional." Thus on Nov. 2–3 the shaded area equalled 6·1 sq. cm., the water flow was 1·005 litres/min., the extra resistance 30 ω, and the ratio R was equal to 1·33 estimated by proportional parts to the mid-hour of the experimental period considered. Performing the multiplication we have:

$$6{\cdot}1 \times 1{\cdot}005 \times \frac{14{\cdot}4 + 30{\cdot}0}{14{\cdot}4} \times 1{\cdot}33 = 25{\cdot}1,$$

which is the muscular energy proportional as shown in the last column but one of Table V.

The total muscular energy proportional, reckoned in square centimetres, gives the energy of muscular effort in Calories, when it is multiplied by the heat value of 1 sq. cm. of muscular energy proportional and by



Nov. 2nd 1925
Nov. 3rd 1925
Zero of Galvanometer
Nov. 3rd 1925
Nov. 4th 1925
Nov. 4th 1925
Nov. 5th 1925
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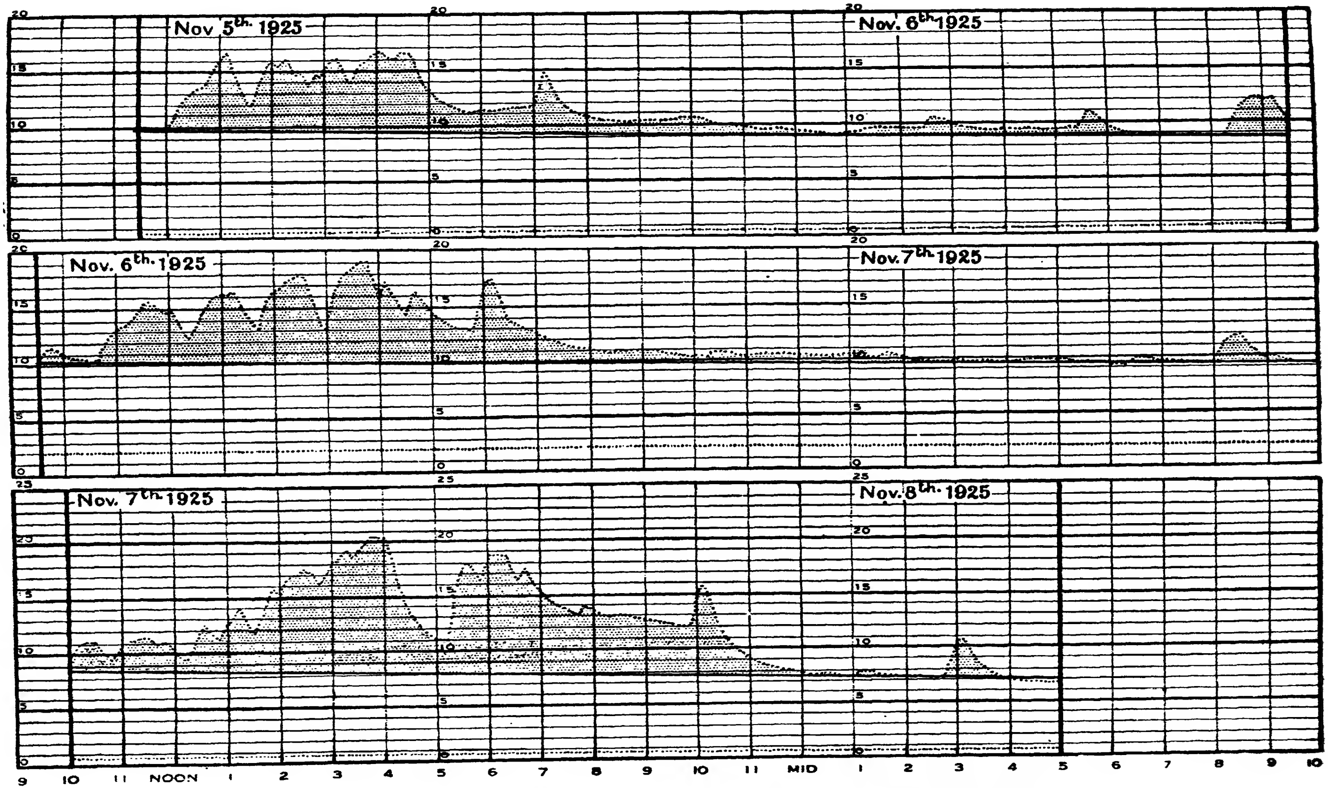

Fig. 8. Galvanometer charts, showing heat removed in the water stream during the experiment with the Berkshire pig, ending Nov. 8, 1925.

the factor $\frac{133}{130}$ to allow for the fact that although the experiment lasted 133 hours, muscular energy observations were only taken over 130 of these. The heat value of 1 sq. cm. of muscular energy proportional will of course depend on the galvanometer, leads and couples employed as also on the diameter and rate of rotation of the drum—in this case it was 4·24 Calories. Performing the above multiplication we find that the total energy of muscular effort for this experiment was 2248 Calories.

Table V. *Computation of "muscular energy proportional"* (*Berkshire pig, Nov.* 2–8, 1925).

Chart date 1925 (Nov.)	Shaded area cm.²	Muscular energy estimation		Total hours estimated	Mid-hour of estimation	Observation time	Water flow	Extra resistance ω	Ratio R		Muscular energy proportional cm.²	Hours fasting at observation time
		Begins	Ends						At observation time	Interpolated to mid-hour		
2–3	6·1	5.0 p.m.	10.0 a.m.	17	1.30 a.m.	3.30 a.m.	1·005	30	1·34	1·33	25·1	11½
3–4	10·8	10.0 a.m.	9.30 a.m.	23½	9.30 p.m.	2.30 a.m.	0·995	30	1·45	1·42	47·0	34½
4–5	24·7	10.0 a.m.	10.0 a.m.	24	10.0 p.m.	2.30 a.m.	1·005	30	1·89	1·81	138·5	58½
5–6	19·5	11.30 a.m.	9.30 a.m.	22	10.30 p.m.	3.0 a.m.	1·000	15	1·68	1·72	68·4	83
6–7	22·4	9.30 a.m.	10.0 a.m.	24½	9.30 p.m.	3.0 a.m.	1·005	15	1·93	1·87	85·9	107
7–8	33·5	10.0 a.m.	5.0 a.m.	19	7.30 p.m.	5.0 a.m.	1·000	15	2·40	2·24	153·1	133
			Totals	130							518·0	133

Similar computations have been made for all the curves of Figs. 1 *a* and 1 *b* with a few exceptions on account of bad lie of the points on the curve making the computation somewhat uncertain, and also in two cases on account of an abnormally high value of R (see p. 163).

Table VI shows the energy of muscular effort and the heat represented by the total shaded area within the thick lines in Fig. 7; for brevity we will refer to this amount of heat, in what follows, by the letter D. Clearly space does not permit full details of all experiments as in Table V. The columns giving the energy under these two heads in terms of Calories per hour are computed by dividing the energy by the time in hours from the beginning of the experiment until the last observation taken, *i.e.* the first observation on the flat part of the curve of descent.

The estimate of the energy of muscular effort for the experiment of Nov. 20, 1926, with the Middle White pig is certainly too low by an appreciable amount as the computation had to be made from the first and third days, the second being rejected owing to a large and unavoidable increase in the water flow, causing a disturbance which rendered the second day's curve useless for this purpose. It could be seen plainly however that the figure should be higher.

No corrections for body temperature variations are made in the above figures although these are in a sense integrals and so ought to receive

such correction, but there are no suitable temperature observations from which to make them and the curves are drawn from observations made at a particular moment which *may* be unaffected by this.

Table VI. *Energy of muscular effort and energy D.*

Date	Pig	Energy D Cals.	Energy of muscular effort Cals.	Mean energy D Cals. per hr.	Mean energy of muscular effort Cals. per hr.	Total Cals. per hr.
26. vi. 25	Berkshire	388	658	6·7	11·4	18·1
17. vii. 25	,,	398	625	6·6	10·3	16·9
8. viii. 25	,,	887	883	10·8	10·7	21·5
29. viii. 25	,,	849	1013	10·1	12·1	22·2
17. x. 25	,,	1212	1450	11·2	13·4	24·6
8. xi. 25	,,	2790	2248	21·0	16·9	37·9
5. xii. 25	,,	2681	3563	25·2	33·4	58·6
30. i. 26	,,	1910	1336	17·8	12·5	30·3
19. xi. 25	Middle White	446	351	7·4	5·9	13·3
11. xii. 25	,,	867	393	10·8	4·9	15·5
10. i. 26	,,	412	642	7·0	10·9	17·9
5. iii. 26	,,	757	1180	9·1	14·2	23·3
2. iv. 26	,,	1025	870	12·3	10·4	22·7
1. v. 26	,,	547	808	9·3	13·7	23·0
11. ix. 26	,,	769	695	13·0	11·8	24·8
20. xi. 26	,,	648	713 +	10·7	11·8 +	22·5 +
9. iv. 27	,,	1866	1763	22·2	22·0	44·2
29. x. 27	,,	1126	1183	18·8	19·7	38·5

Two things strike one about these results—firstly that if the totals in col. 7 were plotted against time or live weight they would show a flat part similar to that in the curve in which fasting katabolism is plotted against time or live weight, and secondly the very fact that some such curve is formed by the sums of two factors differing much more erratically among themselves (more especially in the Middle White) seems to point to the existence of a certain reciprocity between them in the sense that when one increases the other will tend to be less, though this tendency may be masked by the obvious tendency of *both* to increase with age.

Seeking a reason for this, a possible explanation occurred to me which showed the way to a much more clear cut demonstration of the facts than is contained in the foregoing paragraph. It seemed likely that if this reciprocity existed it might be due to the energy D being made up of two parts, one the thermic energy and the other due to variation of nutritive plane. That anything of this nature could take place in so short a time was of the nature of an hypothesis. Now if this were so the tendency for the one factor to increase as the other fell might be correlated with the actual nutritive plane on which the animal found itself

at the time of going into the calorimeter[1]. If this were high it would have a long way to fall, increasing D, and at the same time, being presumably more comfortable, the animal might be comparatively quiet. Conversely if the nutritive plane were low at entry D would be small and the muscular energy developed might be considerable.

If this is so *the effect will be most noticeable in the earlier hours of the experiment*, and here some compromise is necessary since if the first 18 hours (say) were taken the errors in computation of muscular energy would be large. Therefore the first 42 hours was chosen for the test and the energy of muscular effort together with that part of D which fell within the first 42 hours was computed. The figures are given in Table VII and are plotted against age in Fig. 9. They are sufficiently convincing.

Table VII. *Quantities similar to those of Table VI, computed for the first 42 hours only.*

Date	Pig	Portion of energy D included Cals.	Energy of muscular effort Cals.	Mean energy D Cals. per hr.	Mean energy of muscular effort Cals. per hr.
26. vi. 25	Berkshire	387	450	9·2	10·7
17. vii. 25	,,	398	326	9·5	7·7
8. viii. 25	,,	798	282	19·0	6·7
29. viii. 25	,,	756	366	18·0	8·7
17. x. 25	,,	980	521	23·3	12·4
8. xi. 25	,,	2257	324	53·7	7·8
5. xii. 25	,,	2245	1042	53·5	24·8
30. i. 26	,,	1642	408	39·1	9·7
19. xi. 25	Middle White	440	269	10·5	6·4
11. xii. 25	,,	752	250	17·9	5·9
10. i. 26	,,	374	440	9·0	10·5
5. iii. 26	,,	742	432	17·7	10·3
2. iv. 26	,,	956	421	22·8	10·1
1. v. 26	,,	546	623	13·0	14·8
11. ix. 26	,,	768	503	18·3	12·0
20. xi. 26	,,		Second day rejected		
9. iv. 27	,,	1780	1273	42·4	30·3
29. x. 27	,,	1088	1023	25·9	24·4

In *every* case and in *both* pigs the points to the left of the dotted ordinate obey the rule that as the energy of muscular effort increases that under D decreases and *vice versa*. There appears to be no definite quantitative relation between the amount of rise and fall, and it may well be that in future estimations an isolated point may fail to obey the rule, but the agreement here in eleven cases out of eleven during youth appears to exclude any possibility of accident.

To the right of the dotted ordinates the connection between the two

[1] This nutritive plane would depend on other things besides the actual rationing scheme, *e.g.* external temperature, exercise, etc. Of course the suggestion cannot well apply to cases in which the energy D is less than the computed thermic energy, but the difficulty disappears if the suggested modification in theory (p. 175) is accepted.

is much less obvious, if indeed it is present at all. One is therefore constrained to seek a correlation between this and some other of the phenomena of metabolism in youth. This appears to be present to some

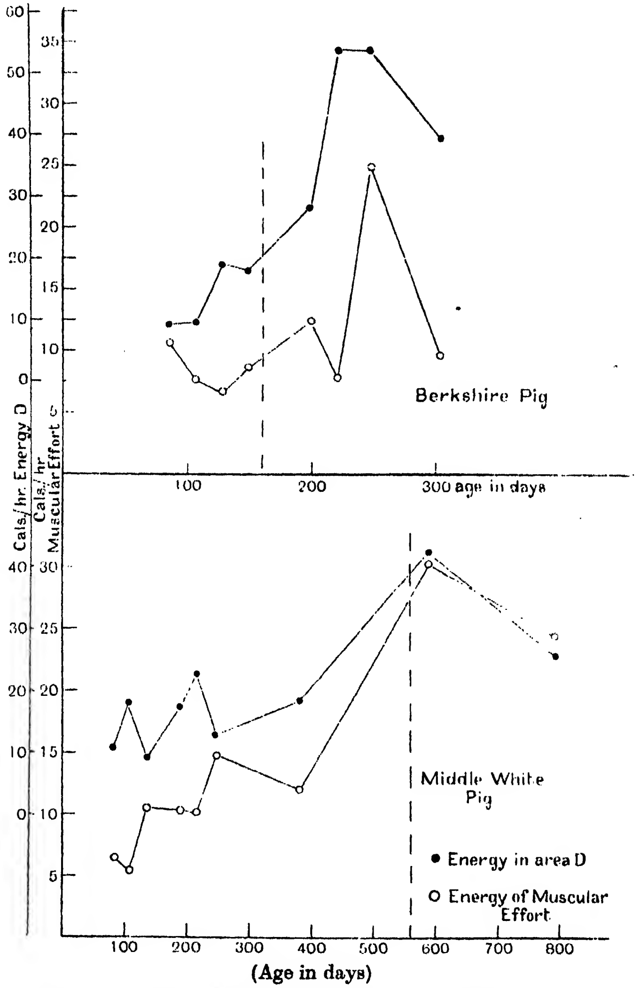

Fig. 9. Reciprocal variation of muscular effort and heat production during youth.

extent in the curves of Fig. 6—the dotted ordinates of Fig. 9 being drawn to correspond in time to that at which the excess metabolism over that required by the surface law has passed its maximum and declined to one-quarter of its maximum value and is of course shortly to vanish altogether. It looks from this as if it might be this factor which is

concerned in the "see-saw" effect observed, and it is difficult to see why the effect should disappear with age if it is *entirely* due to the variation of nutritive plane as suggested above; on the other hand it can hardly be set down entirely to an interchange between the "growth quota" and muscular work, as there then appears no reason why it should be more obvious in the early part of an experiment. Perhaps both factors are at work simultaneously. Anderson and Lusk (27) found some ten years ago that a dog doing a definite amount of work generated appreciably the same amount of heat whether it had previously been fed with glucose or not; Benedict and Murschhauser (28), on the other hand, found no such effect with men. In any case the magnitude of the effects here noted is much more noteworthy than that found by Anderson and Lusk. Schneider, Clarke and Ring (29) have recently found the standard metabolism of 3 out of 5 athletes reduced after training—what bearing this may have on the matter is not clear, it seems contrary to de Almeida's (30) and other results, but I have only seen the paper in abstract.

That an exchange between muscular energy and that part of the fasting katabolism due to growth should take place seems physiologically possible if the view I put forward in a previous paper, that the high metabolism in youth is due to energy wasted in the growth of sarcoplasts in the muscle, is correct; for then, since we know that oxygen is a *sine qua non* for all ordinary growth and also for muscular recovery after contraction (31), may it not be that removal of lactic acid has a preferential claim to oxygen from the blood and that growth may thereby be hindered? That this recovery process has a *very strong* claim to oxygen is evident from the immediate and very large CO_2 production as soon as muscular work is begun.

Rationing.

We must now consider how the foregoing results bear upon the determination of the maintenance ration. If we accept the results we appear bound to reject the fasting katabolism as a figure sufficient in itself for the determination of maintenance requirement since it is clear that the normal nett energy values do not hold in the neighbourhood of the fasting katabolism and possibly with only approximate accuracy in the case of a single animal even in the region of maintenance[1].

[1] In the experiments of Armsby and Fries (*J. Agric. Res.* **3**, 435), taking the same food and the same animal, normal variations in the increment of heat production per kilogram were often of the order of 20 per cent. of its mean value and occasionally even more than this, as *e.g.* in the case of Timothy Hay.

To secure this the following additions must apparently be made to the fasting katabolism in nett energy deduced by the surface law from the adult value per unit area.

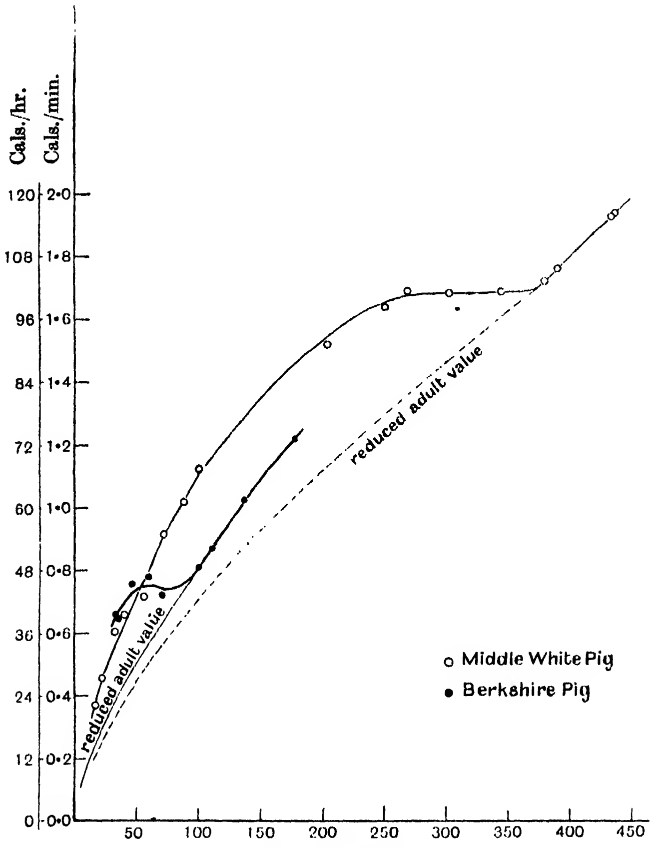

Fig. 10. Variation of fasting katabolism with live weight, showing also reduced adult values.

(*a*) A "thermogenic quota" depending on how much the environmental temperature falls short of the critical.

(*b*) A "growth quota" depending on age.

(*c*) A "quota" due to the supposed change in nutritive plane.

(*d*) A "labile quota" to provide at will extra growth, muscular energy, or extra heat which would appear as an addition to (*c*).

Probably one ought to add a fifth section consisting of muscular energy which is not replaceable as in (*d*) but is either present or absent.

The first needs no comment. It may be supplied as metabolisable energy and in whole or in part by the thermic energy of those quotas which have to be supplied as nett energy.

As regards the growth quota it seems possible that it is to some extent interchangeable with muscular energy, but it is hardly likely to be so entirely. More probably the substantial amount which normally appears added to the fasting katabolism in young animals is not greatly altered in amount by this interchange. The amount of this in nett energy may be read off from the curves in Fig. 10 for the two pigs here discussed.

The quota (*c*) cannot be determined practically since it appears as heat indistinguishable from that due to liberation of thermic energy from those quotas supplied as nett energy, or that appearing in quota (*d*). This latter quota contains by hypothesis only that part of growth energy and energy due to change of nutritive plane which is completely interchangeable with energy of muscular work, and could theoretically be supplied either as nett energy for work production if this were definitely known or it could be converted into heat and estimated in metabolisable energy, but it is unlikely that muscular work could *all* be converted in this way.

We are faced then with two out of the four additions to the fasting katabolism which cannot be determined with certainty[1]. There remains the possibility that though the effects cannot be sorted out yet their sum represented by the sum of the energy D and the energy of muscular effort may be proportional to the ration. In this case if we arrange the various quantities of meal fed in ascending order of magnitude and against each the energy sum mentioned the connection should be apparent, but, as may be seen from Table VIII, it is not.

We appear then to have reached an impasse, but as the theories

[1] If we assume with Armsby that the nett energy required for work production is equal to the body energy metabolised in doing the work, there remains the problem of efficiency in other animals than the horse, and in any case we are in very little better case—since even if the quota (*c*) were the only unknown it cannot be determined by difference since this involves a knowledge of the maintenance ration which it is our aim to determine. The two quantities could presumably be determined together from a series of simultaneous equations if we had a large number of results on each breed of pig at one age and weight but to get any distance by this method would involve team work indeed. It may be added that the energy due to change of nutritive plane could not be determined by subtracting the thermic energy of the food from the energy D since it is uncertain what amounts of feeds before the last should be assumed operative in computing the thermic energy to be subtracted; in addition it is, in view of results described earlier in this paper, very doubtful how far determinations of thermic energy hitherto made can be relied upon generally for constancy in individual cases and more especially with animals approaching the fasting level of metabolism.

hitherto elaborated do in fact work pretty well in practice one is tempted to seek a solution of the riddle in some slight modification of theory which will not seriously disturb the general structure and yet will allow of the new facts being assimilated into it. This the writer believes may be found in the following suggested variation of accepted ideas:

> Specific dynamic action of a *ration* as a phenomenon distinct from change of nutritive plane does not exist, except in so far as it may be due to *true* work of digestion.

Table VIII. *Variation of above energy sum with ration fed.*

(Observations in italics refer to the Berkshire pig.)

Ration lb. oz.	Energy sum Cals.	Ration lb. oz.	Energy sum Cals.	Ration lb. oz.	Energy sum Cals.
1 0	797	2 6	*1770*	3 12	*6244*
1 6	1260	2 6	*1862*	4 0	2309
1 14	1054	3 0	1895	4 8	1464
1 14	*1046*	3 0	1355	4 8	*3246*
1 14	*1023*	3 0	*2662*	5 4	1361 +
2 6	1937	3 0	*5032*	6 0	3629

In other words the rise in metabolism which occurs after food is due to a change of nutritive plane as also is the fall to the fasting level; it is a function of the *animal* and only in a minor or indirect way a function of the *food*, and the fraction of the metabolisable energy which will appear as thermic energy in a single experiment with an animal cannot be expected to approximate *very closely* to the mean—in short, if a large number of experiments were made by the method of Armsby and Fries and the increment of heat production per kilogram determined for a single feeding stuff, the frequency distribution of the deviation from the arithmetic mean would not be normal. Nett energies, in short, are to be regarded as *statistically* rather than *physiologically* constant.

That protein should raise the nutritive plane to a greater extent than carbohydrates or fats is not surprising since in its absence in the food wear and tear as measured by N_2 elimination is reduced to a minimum; consequently any increase of it will presumably increase tissue rebuilding liberating katabolites of considerable energy content, the action of which is additive to that of carbohydrates in the blood[1].

[1] Whether this is really a drug action or otherwise affects only the *means* by which the rise in metabolism is brought about and is immaterial where a normal ration is under consideration, since the observed specific dynamic action of protein is only about three times that of an isodynamic equivalent of carbohydrate (see Rubner, *Gesetze des Energieverbrauchs*, pp. 73 *et seq.*, Leipzig 1902) and the percentage of protein in the ration will not vary enough to make any appreciable difference. In any case the important point is that the change, however brought about, is a change in the nutritive plane and only in the roughest way proportional to the quantity of food in any particular case.

That this action should be slightly augmented in the cases instanced by Aub and Du Bois (32) of an achondroplastic dwarf and a legless man is quite as one would expect, since the fasting katabolism in both cases was below that of the normal controls and the ration therefore presumably equivalent to a larger one, and consequently the nutritive plane would tend to be raised to a greater degree. Again it is easy, on this assumption, to account for the observations of Wang and Strouse (33) who found specific dynamic action to be *less* in obese subjects and *more* in thin ones than in normal controls, the ration being in effect less in the first case and more in the second. These cases are difficult to account for if it is maintained that a definite amount of food contains a definite amount of thermic energy for a particular kind of animal, and that this energy is eliminated, after the food is eaten, appearing as specific dynamic action. Clearly also on the assumption made the facts mentioned on pp. 160 *et seq.* and in Table VIII need no special explanation, whereas they appear to contradict accepted theory.

That carbohydrates should raise the nutritive plane more than fats is also comprehensible since the active mass will probably be smaller at any moment in the case of fats, due to digestion proceeding largely by chemical action on the *surfaces* of globules constituting an emulsion.

There seems to be no evidence of any lowering in the nutritive plane, such as is produced by prolonged feeding on submaintenance rations, ever having caused the standard metabolism of the experimental animals to fall below the normal fasting katabolism (34), as seems quite possible if this fall is independent of and takes much longer to make itself felt than that which takes place in fasting. The test in this case would of course be to fast an animal which had already been on a submaintenance ration for a considerable time. The metabolism in both animals and men on submaintenance rations appears, as far as present evidence goes, to fall down as far as, but not below, the fasting level.

For rationing purposes then we must apparently be content to regard the animal as a variable system. Where a number of animals are to be fed, and they are rationed according to some scheme worked out on that particular breed of animal, in terms of nett energy, the computed average gain may be looked for; on the other hand there seems no reason to suppose that if the *breed* is changed the figures will still hold. In the case of a single animal, instead of a number, they might do so over a long period, owing to variations in the energy D balancing as regards extremes and giving a mean value not greatly different from that attained with a number of animals.

For the animals experimented upon the assumption made as regards specific dynamic action enables us to compute rations on a rather different system. The maintenance ration will consist of the following factors which may be computed in terms of metabolisable energy[1].

(*a*) The adult fasting katabolism in accordance with the surface area of the animal.

(*b*) A thermogenic quota if the surrounding temperature is below the critical.

(*c*) A growth quota.

(*d*) A quota allowing for muscular effort and change of nutritive plane together with any part of (*c*) which may be replaceable by muscular energy. This we may call the *metabolic excess*.

The first three may be grouped together and read off from the curves of Fig. 10 for the Berkshire and Middle White at 16·5° C. As regards (*d*) if we take a value for this from a curve run through points plotted from the figures in the last column of Table VI the result will be too small, since these represent the *mean* height of the curve of descent above the fasting level from the beginning till the end of the experiment. To take the height at 12 hours (say) is open to all the objections which have been urged against the standard metabolism. Probably the best result may be anticipated by taking the quota (*d*) as:

m (figure in col. 5, Table VI) + (figure in col. 6, Table VI),

m is a factor, between 2 and 3, which when used as a multiplier gives a figure which added to the muscular energy and the sum of (*a*), (*b*) and (*c*) gives maintenance; it might theoretically be estimated closely by putting in each case

$$m_1 = \frac{\text{Excess metabolism over fasting level at 6 hrs. in Cals./hr.} \times \text{Time to fasting level in hrs.}}{\text{Energy } D}$$

then m would be the mean of the results m_1, but the time taken to reach fasting level is very difficult to estimate accurately enough. In what follows I have assumed $m = 2$ so the results may be expected to be *slightly low*. Using this figure and running a smooth curve through the points obtained as in Fig. 11 we have a curve from which the metabolic excess may be read off.

The deduced rationing curves for normal maintenance allowing for ordinary small movements as in a pen or small sty are shown in Fig. 12. They are prepared by adding together the curves of Figs. 10 and 11.

[1] The apparent advantage of this is illusory since the larger the thermic energy of the feed (using the term with its usual significance) the larger will the metabolic excess, see (*d*), become.

Similar computations have not yet been made for the Large White pig and a new set of records may well be necessary for this purpose as the ones at present available are far less perfect than those appertaining to the other two breeds. As an approximation it *may* be legitimate to argue that the curve for the Large White corresponding to those in Fig. 11 will bear an analogous relation to that published five years ago (*l.c.*) to what these curves bear to those of Fig. 10. Up to 150 lb. weight at any rate there seems to be little against adopting the mean of the Berkshire and Middle White values as a temporary expedient. With all reserve then, the corresponding curve for the Large White is sketched in up to 150 lb. weight in Fig. 12. All metabolisable energies are reduced to nett

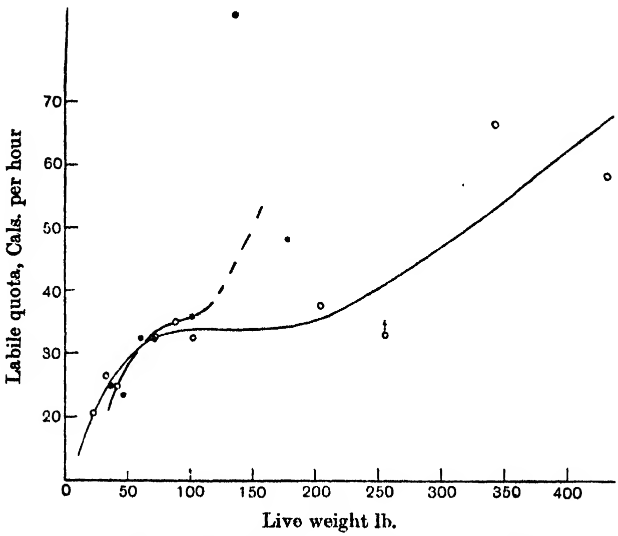

Fig. 11. Auxiliary curves for estimating the metabolic excess.

energies for this curve by multiplying by $\frac{80}{100}$ *to make it easier of application to other meal mixtures.*

The interest of these curves centres in the question of their applicability. Determined in each case on one animal, have we any reason to think that the differences indicated between the breeds may be more than casual variations of individual animals? In human beings the investigations of Benedict (35) have shown normal variations of a number of individuals of one weight and size to be ± 10 per cent., while Magnus-Levy (36) found similar deviations on one man at different times of about the same order. If therefore a long series of experiments with a man

were made, one would expect to be able to draw a line of mean metabolism through the observation points, and that the individual points would depart from this to the extent of 10 per cent. on either side and

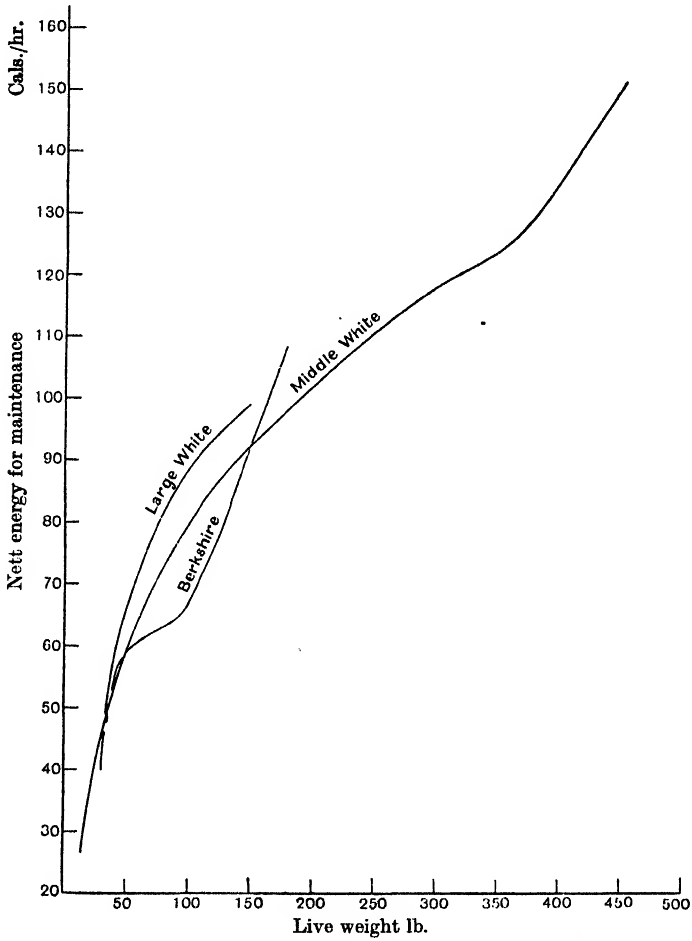

Fig. 12. Rationing curves in terms of nett energy to provide maintenance at different weights for three breeds of store pig when the animals are living a normal life in a small sty or pen at 16·5° C.

only variations in the height of the mean line would be significant from the point of view of the general trend of his metabolism. Now since the variations on taking a number of similar men on one day are only of the same order, it is clear that in man there cannot be any very great varia-

tions in this mean as between individuals of the same race. In our experiments with the pigs the deviations of the individual results are considerably less than 10 per cent. probably owing to the practice of taking the fasting katabolism rather than the standard metabolism so that erratic variations of one individual from time to time are evidently small. Between individuals of the same breed some differences may occur, and, while evidence is largely lacking, it seems unlikely to amount to as much as the observed differences between the breeds shown in Fig. 12, and here the matter must for the present be left.

With the above reservations it appears that the Berkshire pig if kept in a pen at 16·5° C. costs less for maintenance between 50 and 150 lb. than either of the other breeds, the Middle White occupying an intermediate position. For other conditions as regards temperature and approximately as regards exercise the appropriate curves are computable, but this clearly finds no place in a scientific discussion of the matter being purely mechanical.

On p. 157 attention has been called to deductions from this research concerning the size of litters, and that this does represent common experience is abundantly clear from reference to numerous books on the practical side—one may perhaps quote Morrison (37):

"The Berkshire...breed is noted for quick fattening and early maturity. The sows are gentle in disposition and make good mothers. The litters are not large, though by watching, feeding and management of breeding stock there should not be much to complain of. Better to have a breed which farrows a litter of 9 or 10 healthy youngsters and rears them to a uniform weight, than a litter of 15 to 17 of nondescript sizes of which half die or have to be killed because there is neither teats nor milk to nourish them."

In the same volume the author states that the Middle White is not quite so prolific as the large white.

The points as to quick fattening and early maturity are deducible from the results of the foregoing experiments if nett energy for production and appetite are the same in each breed.

The advantage that the Middle White enjoys over the Berkshire in better toleration of adverse weather conditions may well be due to its low critical temperature if this should prove to be general with the breed.

For some purposes it may be more to the point to refer the data to age rather than weight, and if this is done in the two pigs under discussion there is little to choose between them between 100 and 300 days. The Large White still remains well above them in maintenance requirement.

The extraordinarily diverse findings in experiments with these breeds (38) may be due in part to this different standard of reference, and again where the computation is of lb. of meal per 100 lb. gain or the like, it clearly depends between what two weights the 100 lb. gain is taken. It seems possible that a careful analysis of the results in the literature might repay the trouble.

ACKNOWLEDGMENTS.

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SUMMARY.

The metabolism of a Berkshire and a Middle White pig has been investigated by means of the calorimeters at the School of Agriculture, Cambridge. The general routine and technique of the observations have been as heretofore.

Measurements of the fasting katabolism of each of the two pigs have been obtained in a series extending from an early age to maturity, and the phenomena in general follow the lines of those originally discovered in the Large White; but the fasting katabolism of the Middle White was below that of the Large White earlier studied.

The fall in body temperature and in metabolism during the fasts were found to be correlated, and the possible effect of skin colour in this matter is noted.

The effect of environmental temperature is investigated and reasons are given for supposing that the critical temperature of the Middle White pig is very low.

It is concluded that the existence of a maximum somewhere in the curve showing fasting katabolism per unit area at different ages is

necessitated by the two physiological facts (*a*) that warm blooded animals have to be maintained at a temperature which varies only within very narrow limits, and (*b*) that the processes of growth are accompanied by waste of energy as heat.

The fasting katabolism per unit area in pigs is shown to be greater than in several other animals including man, and this is ascribed to absence of an insulating coat.

Thermic energy is discussed in relation to the actual specific dynamic action manifested on various rations in the calorimeters and to the energy expended by the animal in muscular effort during the course of an experiment. It is shown that there is during youth a reciprocal interchange between some of the energy devoted to muscular movement and that which is wasted as heat; the effect is ascribed in part to the animal being less restful as it gets on to a lower plane of nutrition and partly to a preferential demand for blood O_2 on the part of the processes of recovery of potential energy in the muscle, over that of the growth processes.

When room has been found for the above facts in current theory it is shown to be difficult or impossible to determine a maintenance ration with precision. A modification is suggested in the sense that the distinction between specific dynamic action and change of nutritive plane is to be abandoned, the two being combined. This is shown to fit in well with the results of independent investigations on specific dynamic action and at the same time allows of the computation of maintenance rations for a pig of one of these breeds living a normal life in a pen.

Reasons are given for thinking that differences observed are for the present to be regarded as peculiar to the breeds of pig and not as due to individual differences.

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THE ACTION OF CERTAIN CHEMICAL SUBSTANCES ON THE ZOOSPORES OF *PSEUDOPERONOSPORA HUMULI* (MIY. ET TAKAH.) WILS.

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During the summer of 1928 a series of experiments was carried out with the object of testing the suitability of various substances for use on Hops to combat attacks of the Downy Mildew (*Pseudoperonospora Humuli*).

The first experiments were begun early in June. Young hop plants growing in pots were chosen and pairs of leaves at the same node selected. One leaf of each pair was sprayed, using an atomiser, and, when dry, was inoculated with drops of water containing zoospores of the fungus; the other leaf at the node was used as a control and the same inoculum placed on it. In order to provide an atmosphere in which the inoculation drops would not immediately dry up, the plants were placed in a damp chamber[1] erected in a well-lighted and unheated glasshouse. It was considered that the ultimate requirement of a protective spray was that it should prevent the entry of zoospore germ-tubes into the leaf or hop-cone to which the zoospores would be carried by rain.

The first substance to be tested on the growing plant was a proprietary brand of colloidal sulphur, "Ialine[2]," to which 0·5 per cent. saponin was added to secure an unbroken covering of the spray deposit. At the same time, to allow of the effect of the spray material on the zoospores being observed under the microscope, watch glasses were lightly sprayed with the same liquid. After the leaves and the watch-glasses had dried for 24 hours the former were inoculated and the following tests were made with the watch glasses, using the same inoculum.

A drop of water containing the zoospores was placed on the dry spray deposit in one watch glass and a similar drop on the surface of a clean watch glass. It was found that the zoospores, at first actively moving, were unable to exist for more than a minute in the drop of water in the glass sprayed with colloidal sulphur and saponin, whereas in the clean glass the movement was unchecked.

This somewhat surprising result led to a trial of the separate constituents of the fungicide and it was found that neither colloidal sulphur

[1] This measured 12 ft. × 6 ft. × 7½ ft. high, and was made of muslin kept wet by constantly dripping water.

[2] The same brand was used in the subsequent experiments with colloidal sulphur.

alone nor the finest ground sulphur alone, when added to drops of water containing zoospores, had any immediate or strongly inhibitory effect on the activity of the zoospores. It was therefore concluded that the saponin, in addition to acting as a spreader, was actually the fungicide, and one trial of this substance at a strength of 0·5 per cent. showed that it instantly killed all zoospores and caused them to disintegrate in a few seconds, presumably by inducing changes in surface tension. Preliminary trials with soft soap showed that zoospores were similarly unable to exist in 1 per cent. and 0·5 per cent. solutions. The effect was immediate stoppage of movement and a rounding off of the zoospores which either went to the bottom or rose to the surface of the drop. There was then a slow or rapid swelling according to the strength of the solution, and finally a sudden disintegration. Thus, by the addition of a trace of saponin or soap solution, a drop of water with thousands of zoospores in active movement could be immediately cleared of all signs of life.

The rapidity and completeness with which the zoospores were destroyed in the watch-glass tests indicated that the plants sprayed with colloidal sulphur and saponin would not become infected. Of the plants, ten in number, which had been sprayed, the majority showed the first symptoms of infection on the second day on the control leaves, in the form of a yellowish coloration and a glossy appearance of the upper epidermis. After eleven days, when the experiment terminated, all ten unsprayed leaves were heavily infected and bore dense masses of conidiophores. Two of the sprayed leaves each showed a minute area of infection with a single tuft of conidiophores measuring 1 mm. in diameter; the remaining eight leaves were quite healthy and had been efficiently protected by the sulphur and saponin mixture.

The results of this experiment with the living plant and with the watch-glass tests indicated that the fungus is extremely vulnerable in the zoospore stage to the action of soap and saponin. Further experiments were then carried out.

Experiments on the Living Plant.

1. On June 23, 40 hop plants were sprayed: 13 on one leaf each with 0·5 per cent. saponin solution; 13 with a mixture containing 0·5 per cent. colloidal sulphur and 0·5 per cent. saponin, and 14 with 0·5 per cent. lime casein. The 40 opposite control leaves were inoculated first, to allow the spray deposit longer time in which to dry. Inoculation of sprayed leaves was not started until 4½ hours after the spraying had been completed and the lower epidermis appeared quite dry. The inoculum was placed on the leaf by means of a small glass tube, and in doing so care was taken

not to bring the tip of the tube in contact with the leaf[1]. It was found when inoculating the saponin-sprayed leaves that the inoculum spread and ran off the leaf immediately; this necessitated inverting every sprayed leaf and securing it by means of a cleft and bent bamboo standing in the earth of the pot. Lime casein did not prove to be such an effective spreader and the liquid stood on the upturned leaf in large drops[2].

The first infection of the control leaves was seen at the end of the third day and the majority of those sprayed with lime casein showed similar symptoms, viz. large golden brown areas visible on both sides of the leaf.

The plants sprayed with colloidal sulphur and saponin were examined on June 27. Eleven of the sprayed leaves were healthy and two showed small discoloured areas in which mycelium was found. Of the corresponding control leaves only one was healthy, three bore conidiophores and in nine mycelium was found. The tedious process of sectioning the leaves to determine the presence of mycelium was subsequently avoided by placing all leaves, when picked, in damp Petri dishes. When so left overnight infected leaves developed dense grey masses of conidiophores[3]. This method was applied in the case of the 14 leaves sprayed with lime casein, and it was found on June 28 that all had become infected by the fungus. Of the unsprayed (control) leaves, however, only ten were infected. In the case of the 13 leaves sprayed with saponin, all remained healthy whereas the 13 unsprayed all became infected. These results are summarised below:

Exp. 1.

	Colloidal sulphur 0·5 % and saponin 0·5 %	No spray	Lime casein 0·5 %	No spray	Saponin 0·5 %	No spray
Healthy leaves	11	1	0	4	13	0
Infected leaves	2	12	14	10	0	13

2. On June 27 thirty plants were divided into three sets of ten. One, or two, leaves of each plant in one set were sprayed with a mixture

[1] The zoospore suspensions were examined and found active at intervals during the 5 hours required to complete the inoculation and arrangement of the 80 leaves and again after inoculation was finished.

[2] In a test such as this the physical property of saponin is directly connected with the fungicidal effect. The few drops of inoculum spread over the leaf would take into solution more of the active substance than would similar drops when placed on a leaf sprayed with lime casein.

[3] Leaves suspected of containing mycelium were those marked by conspicuous discoloured areas. Presumably the atmosphere in the damp chamber in the glasshouse was sufficiently moist to enable the fungus to infect but not always to fruit on the leaves. In all succeeding glasshouse tests no leaf was recorded as healthy at the end of the experiment until it had been 24 hours in a damp dish and had failed to produce conidiophores.

consisting of 0·5 per cent. soft soap and 0·25 per cent. gelatine; in the next set, with 0·5 per cent. soft soap only, and in the third set with 0·5 per cent. soft soap and 0·25 per cent. flour[1]. The opposite leaf at the node was left unsprayed to provide the control. The inoculations were carried out on the same day, using the methods described above under Exp. 1.

The first signs of discoloration were visible on the upper surface of many control leaves on June 29. On July 4, of the 13 leaves sprayed with soft soap and gelatine, only three were healthy, the remaining ten showing a slight infection by the fungus. Only one (unsprayed) control leaf remained healthy; the other twelve were heavily infected. Eleven of the twelve leaves, sprayed with soap only, became infected, and one remained healthy; the result was the same in the case of the controls, though here the leaves were much more heavily infected, often over the whole lamina. Of the thirteen leaves sprayed with soap and flour-paste, six remained healthy and the other seven became slightly infected in very minute scattered spots; three of the control leaves were healthy and ten were thoroughly infected. The results are summarised below:

Exp. 2.

	Soap 0·5 % and gelatine 0·25 %	No spray	Soap 0·5 %	No spray	Soap 0·5 % and flour[1] 0·25 %	No spray
Healthy leaves	3	1	1	1	6	3
Infected leaves	10	12	11	11	7	10

3. On July 5 thirty plants were divided into three sets of ten. A single leaf on each plant of the first set was sprayed with lime sulphur[2] (1 in 60) + soft soap (1 per cent.); of the second set with Bordeaux mixture[3] (0·25 per cent.) + soft soap (1 per cent.), and of the third set with a mixture of glue (1 per cent.) + soft soap (2 per cent.) + sodium poly-sulphide solution[4] (0·25 per cent.).

The inoculation of these and of the control leaves was carried out on the same evening. It was found that the drops of inoculum had dried by the following day on the control leaves and on those sprayed with the glue mixture, but on the others not until the second day. Discoloration of the lamina, the first sign of infection, was seen on some control leaves on the second day, and by the fourth (July 9) nearly all control (unsprayed)

[1] The flour was first made into paste and this was added to the soap solution.

[2] The sample of lime sulphur used throughout these experiments contained 21·34 per cent. polysulphide sulphur.

[3] The formula used was: copper sulphate 10 gm., hydrated lime 15 gm. and water 400 c.c., *i.e.* 0·25 per cent. Bordeaux mixture.

[4] The stock solution contained 4·2 per cent. polysulphide sulphur.

leaves were showing light patches above, and were black with conidiophores below. The leaves sprayed with the glue mixture were nearly all normal and healthy; several of those to which lime sulphur and Bordeaux mixture respectively had been applied were slightly mottled as though the fungus had penetrated. No conidiophores, however, were visible, and it was therefore concluded that the markings might perhaps be the result of spray injury, especially because other of these sprayed leaves were smaller than the corresponding control at the same node.

On July 9 all leaves were removed and were placed in damp dishes until the next day when it was found that all ten leaves sprayed with the lime sulphur + soap mixture were free from infection; eight of them, however, were either mottled or were smaller than the controls, showing that they had been adversely affected by the spray. Only one of the ten unsprayed leaves remained healthy; the other nine were heavily infected, up-curling, and black with conidiophores below. All of the ten leaves sprayed with the Bordeaux mixture + soap were healthy, but they were mottled with paler areas in which, on sectioning, no fungus could be found. The ten unsprayed leaves were heavily infected. All those sprayed with the glue mixture were free from infection, but six showed slight brown scorching injury at the extreme apex of the lamina. The sprayed leaves were all heavily infected. The results are summarised below:

Exp. 3.

	Lime sulphur (1 in 60) and soft soap 1 %	No spray	Bordeaux mixture 0·25 % and soft soap 1 %	No spray	Sodium polysulphide solution 0·25 % and glue 1 % and soft soap 2 %	No spray
Healthy leaves	10	1	10	0	10	0
Infected leaves	0	9	0	10	0	10

4. On July 9 one leaf on each of ten plants was sprayed with a mixture of lime sulphur (1 in 100) + soft soap (1 per cent.) and one leaf on each of another ten plants with a mixture of lime sulphur (1 in 100) + gelatine (0·5 per cent.). It was found during the process of inoculation that the water stood in drops on the soap and lime sulphur deposit and spread only very slightly on that of the gelatine and lime sulphur. On the following day all drops were still visible and the control leaves were still moist on the lower surface. On the second day (July 11) a few leaves were seen to be moist, but the majority of the drops of inoculum had dried; the first signs of discoloration of the upper epidermis were appearing on the control (unsprayed) leaves.

On July 14 all leaves were removed and were placed in damp dishes

for examination on the following day when all ten leaves sprayed with lime sulphur and soap were free from infection, but all looked paler in colour than other leaves on the same plants. Six of the ten were slightly smaller than the opposite controls, an effect for which the spray must have been responsible. Only one of the unsprayed leaves remained healthy, the remainder being heavily infected. Three of the leaves sprayed with lime sulphur and gelatine showed on the upper surface minute brown areas which were grey with conidiophores below. The remaining seven were healthy, but three showed light-coloured areas on the upper surface. All of the ten unsprayed leaves were infected, black masses of conidiophores extending over most of the lamina. A summary of these results is given below:

Exp. 4.

	Lime sulphur (1 in 100) and soft soap 1 %	No spray	Lime sulphur (1 in 100) and gelatine 0·5 %	No spray
Healthy leaves	10	1	7	0
Infected leaves	0	9	3	10

With respect to the four preceding experiments the substances chosen for use as protective sprays were in all cases spreaders. In each experiment other substances were added which it was hoped would hold the active agent and make it less readily removable; they had been found useful "stickers" by the watch-glass trials described below, and it was essential that they should be tested on tender leaves of the hop plant in order that any possible injury might be observed. In Exp. 1 no injury resulted from the use of colloidal sulphur as a sticker for saponin. Lime casein, a spreader, proved to be inefficient, whereas saponin used alone provided complete protection against the zoospores. As mentioned above (footnote, p. 187), it is possible that efficiency in spreading the inoculation drop may be connected with the fungicidal power of the spreader. As regards Exp. 2, the general failure to prevent infection by soft soap with or without a sticking agent may be considered as due to the fact that the original concentration of the spray (0·5 per cent.) in relation to the comparatively large drops of inoculum was probably insufficient. It must be remembered, however, that both in the preliminary experiment and in Exp. 1 saponin proved completely effective when applied at the same strength (0·5 per cent.). It is possible that during the length of time taken to complete the inoculation a small number of zoöspores might have become rounded off preparatory to germination, and it is not known whether in this state they would be affected by the soap or saponin. In Exp. 3 lime sulphur, Bordeaux mixture and sodium polysulphide were all used as holders for the soap, but their

direct action as fungicides must be taken into consideration. Exp. 4 was designed to compare lime sulphur and soap on the one hand with lime sulphur alone on the other, but it was found that lime sulphur (1 in 100) would not spread sufficiently well. Gelatine as an inactive spreader was consequently employed.

In all these experiments the sprayed leaves were held inverted by means of split bamboos or by wires until the drops of inoculum had dried. The control leaves were not so treated because in the absence of a spreader the water remained sufficiently long on the lower surface without dripping off.

Laboratory Experiments.

Trials were made with various substances to discover whether they were capable of killing zoospores (see (1) below). Wet tests were made (see (2) below) with equal parts of diluted solutions and of the zoospore suspension. It was necessary in most cases, owing to the ready solubility of the fungicidal substances, to find some means of holding them and delaying their solution in water. With this object certain other substances were mixed with them and the liquid either tested immediately or sprayed or dropped on to glass and allowed to dry. In the latter case a standard quantity of the zoospore suspension was applied and removed, the process of application and removal being repeated until a weakening of the killing power was observed. In these tests (see (3) below) the spray deposit was not allowed to dry in the intervals between removal and fresh application of zoospore-containing water.

Some of these tests were also applied to the zoospores of *Phytophthora infestans*.

In all cases control drops from the same pipette were under observation and the zoospores invariably remained active.

(1) *Preliminary trials.*

The following substances were roughly tested in the ways indicated: *sodium silicate, lime casein, colloidal sulphur* and *lime water*. A 0·5 per cent. solution of each of the first three named was used. The liquid was sprayed on a watch glass and allowed to dry. Drops of water containing zoospores were placed on the dry deposit and it was found that neither sodium silicate nor lime casein had any immediate effect. The colloidal sulphur caused some zoospores to come to rest, but many continued to move. A wet test with colloidal sulphur, in which a drop (0·1 c.c.) of a 1 per cent. suspension was diluted to 0·5 per cent. by mixing with an equal drop of water containing zoospores, showed that the zoospores moved about amongst the particles for 12 minutes and then stopped;

many remained motionless but were moving their flagella. A similar test with 0·5 per cent. lime casein was carried out. The zoospores rounded off and then burst after a pause of a few seconds, but diffusion of the active agent into the added drop of water was so slow that an accurate estimation of the time required could not be made. In a watch-glass test with the dried-on deposit of limewater it was noticed that the zoospores were attracted by small fragments at the bottom of the drop. They collected round one or two fragments and remained inactive. *Glycerine.* A trace of this substance added to a drop of water containing zoospores caused instant shrivelling of the zoospores. *Iodine.* By placing a glass bottle-stopper moistened with iodine solution one inch or more away from the microscope stage bearing a slide with a small drop of water with active zoospores, the latter were instantly brought to the surface of the drop where they came to rest and rounded off. This effect was apparently dependent on a current of air sufficiently strong to carry the vapour in the right direction. Iodine in solution (iodine 1 gm., potassium iodide 2 gm., water 300 c.c.) was tested by making 1 c.c. up to 100 c.c. by addition of water. 1 c.c. of this weak solution (0·003 per cent.) was diluted to half strength by addition of an equal volume of zoospore suspension. At this concentration of iodine (0·0016 per cent.) all movement ceased within 5 minutes. The stoppage, which was mostly instantaneous, was followed by slow swelling, and after 10 minutes by bursting of the zoospores. *Bromine.* The extreme sensitiveness of the zoospores to bromine (as to iodine) is shown by the fact that the small amount of bromine vapour which escaped from an ordinary glass-stoppered bottle placed about 5 in. away from the microscope stage was able to arrest and cause disintegration of the zoospores in a drop of water on a slide. *Gelatine.* At a concentration of 0·5 per cent. no effect was produced on zoospores. *Flour paste.* One drop of paste (5 per cent. flour) was added to two drops of inoculum with no effect. "*Sunoco.*" This commercial preparation of a self-emulsifying petroleum oil, at a strength of 0·5 per cent., did not inhibit movement completely but reduced the activity of the zoospores very considerably. *Resin soap.* In a 2·5 per cent. solution zoospores were caused to burst, but 4 minutes were required for their total disappearance. *Caustic soda.* A 0·5 per cent. solution brought about immediate bursting. *Bordeaux mixture.* A wet test, in which 0·125 per cent. Bordeaux was used, resulted in instant stoppage of movement and rapid disappearance of zoospores. The cloudiness of the liquid made observation difficult. *Lime sulphur.* A 0·5 per cent. solution caused immediate stopping and rounding off at the centre of the drop. Zoospores collected in large groups. Particles of free sulphur

coming out of combination in the liquid made it impossible to see the subsequent behaviour of the zoospores.

(2) *Further tests.*

Soft soap. Tests were made at eight different concentrations from 0·5 per cent. to 0·005 per cent., and from the results which are summarised below, it appears that solutions stronger than 0·02 per cent. are completely effective. Reduced activity was immediately evident in all cases. In all dilutions except 0·005 per cent., total stoppage of movement was instantaneous, but in this lowest dilution, 15 minutes elapsed before all zoospores had come to rest.

Effect of soft soap solutions on zoospores.

Percentage soft soap in solution	Swelling	Bursting: Begins	Bursting: Ends
0·5	Instant	Instantly	Instantly
0·25	,,	,,	,,
0·125	Gradual	In few seconds	In few seconds
0·0625	,,	,,	,,
0·03125	Slower	,,	,,
0·0156	Slow	,,	In 60 seconds
0·01	,,	In 60 seconds	In 3 minutes
0·005	Very slow	In 6 minutes	In 2 hours

Caustic soda. Zoospores in a solution containing 0·05 per cent. NaOH were affected as they were in soap solutions and all had disintegrated within 60 seconds. In a weaker solution, 0·025 per cent. NaOH, movement was stopped within 60 seconds, a general bursting followed and all zoospores had disappeared in 10 minutes. *Lime sulphur.* Zoospores in 0·25 per cent., *i.e.* 1 in 400, lime sulphur were caused to stop in 30 seconds and to round off at the surface of the drop. Swelling was slow and bursting only started after 8 minutes; in a 0·125 per cent., *i.e.* 1 in 800, solution the stoppage of movement took place in 30 seconds and, as in the former case, the majority rounded off in large groups at the surface of the drop. Even after an hour bursting was not complete, many rounded-off zoospores remaining on the surface. A wet test with a mixture of lime sulphur and soap was carried out and in a solution containing lime sulphur 1 in 400 and soft soap 0·25 per cent.[1] swelling of the zoospores and finally bursting was very slow. In a solution of half this strength (lime sulphur 1 in 800 and soap 0·125 per cent.) the zoospores all rounded off in 5 to 10 seconds. Swelling was gradual and bursting started after 5 minutes. All zoospores had completely disappeared in 10 minutes from the start of the test. This time is considerably more than

[1] The original solution, before dilution, was prepared by mixing 2·0 per cent. soap solution and 1 in 50 lime sulphur solution in equal quantities.

was required in a solution of soap only (0·125 per cent.) as described above, and it would seem that addition of lime sulphur reduced its efficiency.

(3) *Adherent mixtures.*

In a liquid containing soft soap (0·25 per cent.) and gelatine (0·125 per cent.) zoospores were caused instantly to disintegrate. The same effect was produced when flour paste (0·125 per cent. flour) was substituted for gelatine. Watch glasses sprayed with a thin film of the above mixtures were allowed to dry and were then filled with water and emptied three times, after which drops of water with zoospores were run on. The flour mixture was inactive. The gelatine mixture, however, caused slowing up but no killing for about 5 minutes. At that time zoospores at the bottom of the glass swelled up and some burst, but after an hour no more than approximately one-third of the original number had disintegrated. Soft soap alone did not prove to adhere sufficiently well to the watch glass. A drop (unmeasured) of a 1 per cent. solution, when allowed to dry overnight, killed the zoospores in a drop of water (0·1 c.c.) which was placed on the deposit, but when, after this had been drained off, a second drop containing active zoospores was placed on the watch glass no strong fungicidal effect was produced.

A drop (unmeasured) of a solution containing equal parts of 1 per cent. soft soap and 2 per cent. glue was allowed to dry on a watch glass. Zoospores in a drop of water (0·1 c.c.) applied to the deposit stopped movement in 3 minutes and all burst at the end of 10 minutes. After removal of this drop a second was added and a more rapid action followed; movement was instantly stopped and a general bursting of the zoospores began after 5 minutes. In a third drop movement ceased after 3 minutes and the majority of the zoospores had disappeared after 6 minutes. In the fourth drop added the zoospores continued movement for 10 minutes and were not affected even after one hour had elapsed.

A single drop of a solution composed of 40 c.c. of 4 per cent. soft soap, 40 c.c. of 2 per cent. glue, 10 c.c. of sodium polysulphide solution (containing 4·2 per cent. polysulphide sulphur) and 90 c.c. water was allowed to dry on a watch glass and the deposit was tested as before. In the first drop there was instant killing; in the second all had burst within 3 minutes; in the third within 6 minutes; but in the fourth there was only a gradual slowing after 4 minutes and most of the zoospores were not motionless until after 7 minutes.

A mixture of casein and soap was next tested by means of a single drop (0·1 c.c.) allowed to dry on the centre of a watch glass (soft soap 20 gm., casein 10 gm., sodium hydrate (10 per cent.) 50 c.c.; the whole

being made up to 100 c.c. by addition of water). In the first drop (0·1 c.c.) of a zoospore suspension placed on the dry deposit the zoospores were immediately killed; this was run off and a second drop added, and total destruction of the zoospores resulted in $2\frac{1}{2}$ minutes; for the third drop $5\frac{1}{2}$ minutes were required for the same effect, and in the fourth the zoospores were still moving after 10 minutes.

0·1 c.c. of a solution of soap (2 per cent.) with ferrous hydrate (5 per cent.) was tested. When the drop (0·1 c.c.) of the zoospore suspension was added the dried-on deposit readily came away from the glass in a coloured stream, but it did not quickly diffuse through the added water. Zoospores instantly burst when they came in contact with the soap stream; the remainder which had rounded off when the drop was first added burst gradually as diffusion took place. The first drop was drained off and another added. In this diffusion was very slow and 7 minutes elapsed before all movement had ceased and bursting of zoospores became general. In the third drop added movement continued for more than 10 minutes and the test was not proceeded with. It appeared that the ferrous-ferric hydrate was a good holder of the soap, making a viscous mixture, but it did not adhere well to the glass.

One drop (0·2 c.c.) of a solution of resin soap (5 per cent.) with no added sticker was allowed to dry on a watch glass. Zoospores in an equal drop of water stopped movement and all burst within 4 minutes. In the second drop movement was stopped after $3\frac{1}{2}$ minutes, but bursting was delayed until 6 minutes had passed; then it took place quickly and all zoospores had disappeared at the 7th minute. In the third drop movement continued for 10 minutes without any signs of slowing down and the test was not continued.

The fungicides used on the living plant (Exp. 3) were tested on watch glasses by allowing one drop (0·05 c.c.) to dry on. Successive drops of zoospore suspension, each 0·05 c.c., were placed on the spray deposit and drained off after some definite effect on the zoospores had been noted. The deposit was not allowed to dry in the interval between removal of one drop and replacement by another. In this way lime sulphur (1 in 60) and soft soap (1 per cent.) was found to bring zoospores to a standstill even in the eleventh drop added. The time required for complete disintegration was somewhat long (14–20 minutes) in all the drops added.

Bordeaux mixture (0·25 per cent.) and soft soap (1 per cent.) was tested to the eighth drop; it arrested movement more rapidly than the lime sulphur, but the time required for complete removal of zoospores was about the same, *i.e.* up to 20 minutes at the seventh drop. The

results of these experiments, using lime sulphur or Bordeaux mixture in combination with soap, are summarised below:

Effect on zoospores.

Spray deposit	Movement stopped	Swelling	Bursting Begins	Bursting Ends
Lime sulphur 1 in 60 and soft soap 1 %:				
Added drop 1	Instantly	Very slow	6 min.	—
„ 2	1·0 min.	„	8 min.	—
„ 3	1·5 min.	„	10 min.	—
„ 4	1·5 min.	„	8 min.	14 min.
„ 5	1·5 min.	„	—	14 min.
„ 6	2·0 min.	„	*	*
„ 7	8·0 min.	„	14 min.	—
„ 8	7·0 min.	„	—	20 min.
„ 9	—	„	—	20 min.
„ 10	—	„	—	14 min.
„ 11	5·0 min.	„	—	—
Bordeaux mixture 0·25 % and soft soap 1 %:				
Added drop 1	Immediately	Slow	—	5 min.
„ 2	„	„	—	7 min.
„ 3	„	„	*	*
„ 4	„	„	—	13 min.
„ 5	„	„	—	11 min.
„ 6	1·0 min.	„	—	14 min.
„ 7	1·0 min.	Very slow	—	20 min.
„ 8	1·0 min.	„	—	15 min.

* Drained off and glass left for half-hour interval.

It is known that salts of aluminium have fungicidal properties (7) and field tests have been made by Kelsall (6) with an aluminium-lime mixture. Wet and dry tests, with and without soft soap, were therefore made on watch glasses with a mixture which had the following composition: hydrated lime 0·25 gm., aluminium sulphate 0·25 gm., soft soap 0·5 gm. and water 100 c.c. This 0·25 per cent. aluminium-lime mixture was first tested without the addition of soap. A drop (0·1 c.c.) was placed in the watch glass and to this was added an equal drop (0·1 c.c.) of the zoospore suspension, making the concentration now 0·125 per cent. aluminium sulphate. The test was repeated five times and on every occasion the zoospores were instantly brought to a standstill and rounded off. Bursting was completed in from 2½ to 5 minutes.

In a similar test repeated three times, using 0·125 per cent. aluminium sulphate and soft soap, as shown above, movement was stopped in 5 seconds and bursting was complete in from 2½ to 4½ minutes.

One drop of the complete mixture, *i.e.* with soap, was allowed to dry on a watch glass for 4 hours. To the dry deposit was added an equal drop (0·5 c.c.) of zoospore suspension. All movement was stopped in 5 minutes at the bottom of the drop where slow swelling then took place;

at the surface, however, movement was continued for much longer (15 minutes). The glass was washed out under running tap water and the white deposit still adhered. After draining off all excess water a second drop (0·05 c.c.) containing zoospores was placed on the watch glass. After a few minutes many went to the bottom but others continued to swim. After half an hour very few remained in movement and their progress was restricted and spasmodic. Many were at the bottom of the drop in contact with the precipitate and were in all stages of swelling and bursting. After two hours all zoospores had disappeared.

Several plants in the Wye College hop garden were sprayed, when fully grown, with aluminium-lime mixture, but the incidence of Downy Mildew on the cones of the unsprayed control hops was so slight in 1928 as to make it impossible to judge the protective power of the mixture on the sprayed cones. It was observed that this mixture on the plants was of a light greyish white colour, that it adhered to the foliage in spite of rains and that it caused no scorching.

LABORATORY EXPERIMENTS WITH ZOOSPORES OF *PHYTOPHTHORA INFESTANS*.

At the end of August the opportunity was taken to test the reaction of zoospores of the potato blight (*Phytophthora infestans*) to soap and saponin solutions as well as to iodine vapour. Potato leaves with patches of blight were collected on August 27 and were kept in a damp atmosphere till the next day to increase the growth of conidia. By means of a camel-hair brush the spores were placed in watch glasses of rain water which had just been collected during a shower. Compared with the rapid germination of conidia of *Pseudoperonospora Humuli* which require about $1\frac{1}{4}$ hours at 19° C., those of *Phytophthora infestans* were slow in producing zoospores; at the same temperature (19° C.) 4 hours elapsed from the time of placing the first spores in water to the emergence of zoospores[1]. Four different concentrations of soft soap were employed, the final dilution of each being determined as in the foregoing experiments by using a drop of standard size (0·1 c.c.) and adding to it an exactly similar

[1] In addition to temperature, other factors may be concerned. Thus in the case of *Plasmopara viticola* it has been found (5) that germination takes place in 1 to 8 hours after placing the spores in water. When nutritive solutions are used, zoospore formation is more active at the greater dilutions; the age of the conidium and its condition resulting from previous existence in a wet or dry atmosphere are also to be taken into account. Thus ripe conidia from fairly dry surroundings form zoospores in 6 to 8 hours, but if previously kept in moist air, they form zoospores in 1 to 2 hours. Conidia kept for 2 or 3 weeks form zoospores only after 24 to 48 hours' immersion in water.

drop of zoospore suspension from a pipette. The results are summarised below:

Phytophthora infestans.

Effect on zoospores placed in various concentrations of soft soap.

Soft soap %	Movement stopped	Swelling	Bursting: Begins	Bursting: Ends
0·125	Instantly	Instantly	Instantly	10 sec.
0·0625	,,	,,	,,	4 min.
0·03125	,,	,,	3 sec.	7 min.
0·0156	,,	Gradually	2 min.	12 min.

A similar test with two concentrations of saponin was carried out. In 0·0625 per cent. saponin the zoospores rounded off within 60 seconds and slowly swelled; bursting was almost simultaneous and was completed after 7 minutes. In 0·0156 per cent. saponin, movement was stopped in one minute; the zoospores then slowly swelled and bursting was spasmodical. At the end of 8 minutes a few zoospores remained.

Iodine affected these zoospores in the same way as those of the Hop Downy Mildew. The stopper of a bottle which contained iodine solution was held 2 inches away from a watch glass on which was a single drop (0·2 c.c.) of water containing zoospores. The effect was immediate and zoospores rose to the surface of the drop and came to rest. They then very slowly swelled and started to burst after 3 minutes.

Discussion of Results.

It is clear from the above experiments that the Hop Downy Mildew at the stage when free zoospores have been produced is extremely vulnerable. The zoospores can be exterminated by means of various substances, viz. soap, saponin, glycerine, iodine, bromine, and aluminium-lime mixture. In the case of soap and saponin, and perhaps other substances, it seems possible that the disintegration of zoospores is caused by changes in surface tension. It is somewhat surprising that the lethal action of these substances, specially of soap, on zoospores has not apparently been recorded[1].

The fungicidal power of soap has, however, been recognised; solutions containing 0·5–1·0 per cent. have been used in vineyard practice in Germany against *Botrytis cinerea* (13), and solutions of 5–7 per cent. are stated to control certain powdery mildews, viz. *Sphaerotheca pannosa* (2) and

[1] The action of chemicals on free-swimming zoospores has seemingly been studied by few observers; Wüthrich (14) records the effects of different solutions of metallic salts and certain acids on the free zoospores of *Phytophthora infestans* and *Plasmopara viticola* Millardet and Gayon (9) studied the effect of lime, copper sulphate, and iron sulphate on the zoospores of *P. viticola*; they remark, "...les conidies et les zoospores qu'elles engendrent sont, à l'égard de ces solutions, d'une sensibilité vraiment prodigieuse."

S. mors-uvae (4, 8). Iodine has been employed for the disinfection of seed oats (12) and bromine for other seeds (1, 11 bis). Experiments by Owen (11) have shown that sclerotia of *Colletotrichum tabificum*[1] are killed after 17 hours' immersion in iodine solutions (0·1 per cent. to 0·45 per cent.). The action of iodine as a disinfectant or as a killing agent in laboratory technique has long been known; the use of iodine vapour for this latter purpose was described by Overton (10) in 1890.

With proof now obtained of the extreme susceptibility of zoospores to soap, which has no disadvantages for use on hops, it is to be hoped that some practical method may be devised whereby this or some such zoospore-killing substance will be utilised during the time (about six weeks) when the cones are developing. At this period great anxiety is experienced by the grower, owing to the fact that the cones may be attacked, turned brown and rendered useless in so short a space of time as two or three days. The ready solubility of soap makes it appear at first sight unsuitable on account of the necessity of replacing it after every shower of rain. This difficulty, which is certainly serious, may possibly be overcome (1) by finding some base capable of holding the soap in a wet spray and making it less readily removed, or (2) by employing a soap-containing dust which on account of its easier application could be put on the hops after every rain, at least during the final week before picking.

The preliminary experiments already made show that a mixture of soap with lime sulphur, for example, has a fairly lasting fungicidal power when tested on glass and was successful in protecting the inoculated leaves of growing plants.

As far as we know there are no soap-containing dusts in use as fungicides or insecticides, but their elaboration should not present any serious difficulty. Although it is well known that other dusts in use as substitutes for wet protective sprays do not adhere so well as the wet sprays, there is a possibility that in the case of the closely crowded and overlapping "petals" of the hop cone a dust might prove as effective as a wet spray[2].

SUMMARY.

1. Both *Pseudoperonospora Humuli* and *Phytophthora infestans* are extremely susceptible in the zoospore stage to the action of weak solutions of soap or saponin. The zoospores are caused to disintegrate suddenly,

[1] Now called *Colletotrichum atramentarium*.

[2] In discussing this subject relative to the Vine Downy Mildew, Delacroix and Maublanc (3) state that copper sulphate powders are inferior to the wet sprays in adherent properties except in the one case where the dusts adhere better to the smooth surface of the grapes.

apparently by changes in surface tension, within 60 seconds, in solutions containing over 0·1 per cent. soft soap. Those of *P. Humuli* are more vulnerable than those of *P. infestans.*

2. The fungicidal action of soap and saponin mixed with certain adherent substances was tested on hop plants.

3. The power of adhesion and the fungicidal efficiency of the mixtures were tested by allowing single drops to dry on the surface of watch glasses and by then adding drops of water containing zoospores.

4. Other substances, *e.g.* aluminium-lime mixture, glycerine, iodine, bromine, were also found to kill zoospores rapidly.

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STUDIES IN CROP VARIATION.

VI. EXPERIMENTS ON THE RESPONSE OF THE POTATO TO POTASH AND NITROGEN.

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(With Four Text-figures.)

IN a previous paper (1) the authors have described in detail the design, statistical calculations, and advantages of a method of field experimentation which, on its theoretical side, is based upon the analysis of variance (2). The method is capable of expansion and elaboration in several directions, and the purpose of this paper is to put on record three further examples of experiments in which the new technique has been employed.

As before, it has seemed to us best to present the details of this method in the form of an actual description of experiments themselves rather than as abstract examples. Such a procedure has in this case an added advantage since the three examples chosen follow one another logically, and were each the result of a realisation of both the limitations and the advantages of prior attempts. A consideration of these trials will, it is hoped, enable the experimenter to appreciate the advantages of planning his experiments so as not only to embody an agricultural question, but also to ensure the most accurate decision possible.

When the time was opportune for applying in practice some of the advances in experimental method then available, two of the most important investigations being carried out in the field at Rothamsted, were concerned with

(1) the qualitative aspect of potash manuring,
(2) the interaction of potash and nitrogen.

The crop selected was the potato, and it was to these investigations that the new methods were first applied. In the first two years the variety was "Kerr's Pink" and in 1927 "Arran Comrade." The qualitative aspect of the investigation gave rise to a design which may be designated Type I.

TYPE I.

The problem at hand was to design an experiment capable of distinguishing the differential effects (if any) of potash applied as sulphate,

muriate, and low grade salt (*i.e.* a sylvenite containing, in addition to potassium chloride, a high percentage of sodium chloride). There were thus three treatments, to which a fourth was added with no potash, this being in the nature of a safeguarding plot to ensure that an apparent equality in the efficacy of the three forms of potash was not in reality due to non-effectiveness and consequent lack of response.

The necessity of a high standard of accuracy to distinguish between equivalent dressings of various forms of the same nutrient together with the smaller number of comparisons attempted, led to the adoption of the Latin square arrangement of plots. The principal features of the design have been described elsewhere (2) but may be repeated here.

Each Latin square experiment contains as many replications as there are treatments. In each row and in each column of the square, each treatment occurs once and once only. It is in this respect that it is a square, for in actual shape it may vary from a true square to a rather pronounced rectangle. The actual allocation of the position of any treatment within its row or column is, apart from this one restriction, determined by chance. It thus follows that for a 4 by 4 Latin square, for instance there are 576 alternative arrangements. An actual experiment is a random choice from the total possible arrangements. Though not completely randomised with respect to plot arrangement, this design possesses complete randomness with respect to the elements of variation used in testing significance. Fig. 1 shows the actual arrangements employed during the two years of the trial. The details of the treatment are as follows:

Basal manuring: Superphosphate 6 cwt. per acre; sulphate of ammonia 2 cwt. per acre.

Potash in the form of sulphate, muriate or low grade salt: The equivalent of 2 cwt. per acre of sulphate of potash.

1925

M 444	P 422	O 173	S 398
O 279	S 439	M 423	P 409
P 436	M 428	S 445	O 212
S 453	O 237	P 410	M 393

1926

M 584·0	S 557·0	O 461·5	P 498·5
S 519·5	P 485·5	M 477·0	O 389·0
P 474·5	O 378·5	S 467·5	M 491·5
O 464·0	M 511·0	P 507·0	S 492·0

Fig. 1. Arrangement of two Latin squares with yields in lb. per plot.

Applying the analysis of variance to this arrangement it is possible to obtain a variance due to:

(1) Treatment.
(2) Position.
(3) Random variation of parallels.

Table I. *Total yields* (1925).

Rows		Columns		Treatments		
1	1437	1	1612	(O)	No potash	901
2	1550	2	1526	(S)	Sulphate	1735
3	1521	3	1451	(M)	Muriate	1688
4	1493	4	1412	(P)	Potash manure salts	1677

Table II. *Analysis of variance* (1925).

Variance due to	Degrees of freedom		Sums of squares		Mean square
Treatments:					
Potash *v.* no potash	1		119,700		119,700
Potash manures	2		475		237
	—	3	———	120,175	40,058
Rows		3		1,740	580
Columns		3		5,841	1,947
Parallels		6		1,995	333
Total		15		129,751	—

Table III. *Total yields* (1926).

Rows		Columns		Treatments		
1	2101·0	1	2042·0	(O)	No potash	1693·0
2	1871·0	2	1932·0	(S)	Sulphate	2036·0
3	1812·0	3	1913·0	(M)	Muriate	2063·5
4	1974·0	4	1871·0	(P)	Potash manure salts	1965·5

Table IV. *Analysis of variance* (1926).

Variance due to	Degrees of freedom		Sums of squares		Mean square
Treatments:					
Potash *v.* no potash	1		20,254		20,254
Potash manures	2		1,278		639
	—	3	———	21,532	7,177
Rows		3		12,055	4,018
Columns		3		3,989	1,330
Parallels		6		2,065	344
Total		15		39,641	—

The positional variance is here of the utmost importance since it can be calculated in two directions, as variance of the rows—*i.e.* variability from top to bottom, and variance of columns (variability from side to side). In this experiment the number of degrees of freedom assigned to each positional factor is three, and since any variation in

column totals does not affect the variation in the row totals, the estimates of the variances of the two are independent, and for the purposes of elimination of positional variances are additive. In other words, a considerable amount more of positional variance can be taken out by using this arrangement as a Latin square than by using it merely as four blocks of four treatments. The actual magnitudes of the variances are given in Tables II and IV.

One point may be mentioned here, which, although not demonstrated by these analyses, is sometimes likely to arise. Treated as a Latin square, the data provide six degrees of freedom for the estimation of error. If the variance of either rows or columns had been sacrificed, there would however have been not six but nine available, and the sums of squares corresponding to the rejected three degrees of freedom would have been absorbed in the remainder sums of squares. The question will sometimes arise as to whether the gain effected by eliminating the sums of squares of either rows or columns will counterbalance the loss entailed by having three less degrees of freedom with which to estimate the random error. In both the examples given it will be seen that even though in 1925 the positional variance of the rows, and in 1926 that of the columns, was small, the error is reduced by eliminating them from the error calculations. But, in cases where this is not so, the fact that the Latin square gives a larger error than blocks with only a one directional variance, has sometimes been held to imply a disadvantage. This is not altogether a fair criticism. One of the merits of the method is the recognition that the arrangement of plots has a real effect upon error estimation, and it makes use of that knowledge. If it were possible to say beforehand that in one particular direction, owing to the absence of a large component of soil heterogeneity in that direction, the positional variance was negligible, then it would be advantageous to use the simpler block arrangement. In point of fact, it is seldom, if ever, with annuals, possible to rely on such a contingency, even where a preliminary uniformity trial has been carried out, and the Latin square arrangement is adopted to make sure that the residual variance, on which hangs the precision of the experiment, shall not be inflated from either source. When, in this way, certain elements of error have been eliminated from the field results, the statistician has no choice but to eliminate them in his estimate of error. To include a portion of these because they make his estimate smaller would be to miss the point of making an unbiassed estimate.

These experiments have one defect which in some cases may be

hard to overcome. The inclusion of the no-potash plot (for the reasons specified) does in a year of pronounced response to potash contribute very largely to the treatment variance. In applying tests of significance, particularly what is known as the z test, significance may be claimed for treatment as a whole which is really due entirely to the one degree of freedom potash versus no potash. A further analysis of the variance due to treatment by separating this component, as in Tables II and IV, will, of course, settle the point, but the need of this precaution, and the possibility of carrying it out, requires, at the present time, some emphasis.

The benefit of the elimination of the disturbing element of soil heterogeneity is clearly seen in Tables II and IV, if the positional variance is amalgamated with the random variance as it would be if the old methods of designing field experiments were followed. Table V shows this advantage in terms of the standard error per cent., and of the precision figure based thereon.

Table V. *Advantage of eliminating a portion of the soil heterogeneity.*

	Soil variation eliminated	With soil variation	Soil variation eliminated	With soil variation
Standard error %	2·4	3·8	1·9	4·0
Precision	17	7	27	6

In 1925 soil variation would have increased the error by more than 50 per cent. and in 1926 by more than 100 per cent. The influence on the accuracy of an experiment which such an increase of error entails is shown by the figure for precision. This value is arrived at as follows. On a precision scale a 10 per cent. error, the approximate error of a single plot with many crops, is assigned the value 1 and a 1 per cent. error then has a precision value 100, this latter being the value at which with our present resources it is reasonable to aim. The precision index I will then be

$$I = 100\left(\frac{\sigma}{m}\right)^2,$$

where σ is the standard deviation of the mean yield of each treatment, and m is the mean yield of all.

Type II.

The Latin square form of experiment answered admirably for the foregoing qualitative distinctions between potash manures, but was unsuited to the investigation into the quantitative relationships between potash and nitrogen.

To serve any useful purpose, this had to include several increments of potash with corresponding increments of nitrogen combined in as many ways as the size of the experiment would allow. To have carried out an experiment involving so large a number of treatments in the form of a Latin square, would have been very wasteful of space and effort. Past a certain point with the Latin square the increase in replication does not bring about a decrease in error commensurate with the labour involved. There are indications that comparisons of more than seven treatments or varieties can be made more precisely with other arrangements. Accordingly, when in 1925 twelve treatments were contemplated, positional variance was eliminated by assigning each treatment to each of four similar blocks, the arrangement of which was substantially the same as that of the top dressing series previously referred to (1). Within the blocks, however, the arrangement of the plots was not a random one.

M 491	N 328	C 340	R 508	D 388	A 322	Block I
L 437	J 217	Q 487	P 464	T 272	S 516	
P 450	S 464	R 461	C 320	N 298	M 482	Block II
T 252	D 352	A 281	L 438	Q 515	J 315	
C 341	P 439	S 456	M 466	J 247	N 344	Block III
T 226	L 393	D 338	R 519	A 198	Q 501	
M 449	A 191	N 185	P 472	D 342	T 234	Block IV
Q 461	R 475	J 157	L 377	C 298	S 441	

Fig. 2. Quantitative experiment of 1925, yields in lb. per plot.

The actual arrangement of the blocks as shown in Fig. 2 was determined by the knowledge that there is a high correlation between adjacent plots.

In 1926 a similar experiment was carried out (Fig. 3). The actual

treatments involved in these trials are shown in the following plan where the letters indicate the treatments employed.

1925

Sulphate of ammonia, cwt.	Sulphate of potash, cwt. 0	2	4	6
0	A	C	D	—
2	J	L	M	—
4	N	P	Q	R
6	—	—	—	S

1926

Sulphate of ammonia, cwt.	Sulphate of potash, cwt. 0	1	2	4
0	A	B	C	D
1	E	F	G	H
2	J	K	L	M
4	N	O	P	Q

N 332·0	J 302·5	F 383·0	A 317·5	D 439·0	O 533·5	K 544·5	A 404·5
K 444·5	Q 568·0	O 450·0	D 381·5	L 483·5	B 308·0	F 434·0	N 468·0
B 363·0	C 368·0	M 449·0	L 471·5	H 422·0	P 500·0	G 402·0	E 318·0
H 447·5	E 314·0	P 527·0	G 434·5	M 504·0	Q 561·5	C 356·0	J 456·0
A 351·5	L 495·5	J 443·0	C 383·5	P 559·0	Q 550·0	B 359·0	E 395·5
K 472·5	B 367·5	G 455·5	O 502·5	C 328·5	H 390·5	J 483·0	O 512·0
E 357·5	F 381·5	Q 531·0	D 316·0	N 522·0	M 444·0	A 325·0	D 259·0
N 385·5	H 354·0	P 496·5	M 474·5	F 410·5	G 351·5	K 430·0	L 394·5

Fig. 3. Quantitative experiment of 1926, yields in lb. per plot.

It was realised that the 1925 experiment was inadequately designed with respect to the treatments included, and the distribution of plots within the blocks, consequently in 1926, by using every possible combination and a strictly random arrangement, the experiment was greatly improved. The analyses of variance are set out in Tables VI and VII and the plan of the arrangements in randomised blocks in Figs. 2 and 3.

Table VI. *Analysis of variance*, 1925.

Variance due to	Degrees of freedom	Sums of squares	Mean square
Treatment	11	464,251	42,205
Blocks	3	22,030	7,343
Parallels	33	34,285	1,039
Total	47	520,566	—

Table VII. *Analysis of variance*, 1926.

Variance due to	Degrees of freedom	Sums of squares	Mean square
Treatment	15	261,497	17,433
Position	3	11,303	3,768
Parallels	45	97,361	2,164
Total	63	370,161	—

The 1926 results call for some explanation. For an experiment in which care has been taken to reduce to a minimum disturbing factors contributing to error, the errors are disconcertingly high. This can be traced to the very small amount of positional variance which has been eliminated. The variance due to position is largely caused by soil heterogeneity, as is also the random variance. The difference between the two lies in the fact that the former is due to systematic changes in fertility affecting whole blocks (inter-block variance), whilst the latter is sporadic in its incidence (intra-block variance). So long as the size of the block is such that the changes of fertility which must occur even in one block are systematic, the variation will be reflected in a large positional variance which is all to the good. If, however, the blocks get so large that within the blocks there is local heterogeneity which is not systematic in incidence, such heterogeneity will increase the remainder or random variance. The question as to how much soil heterogeneity variance makes its appearance in the one or the other sections into which the analysis of variance is divided, depends entirely upon the inter-relation of plot size with block size and the type of soil heterogeneity encountered.

In the present instance it would appear that as only some 10 per cent. of the sum of squares contributable by soil fertility variation is assignable to systematic changes, the blocks have been too large to fulfil their function. Greater replication of smaller blocks would have improved the experiment.

It will be noticed that every experiment of this type really constitutes a sort of uniformity trial in addition to answering the normal agricultural purpose. From a number of experiments carried out on one field over a variety of seasons, a very much fuller knowledge of the behaviour of the field is obtained than could be gained from a similar series of the older type.

Type III.

The failure to realise the standard of accuracy desired in the 1926 experiment led to further discussion of experimental design and the evolution of a further elaboration. The simple expedient mentioned

above of increasing the replication in order to ensure greater accuracy, was not possible on the potato crop. To have done so would have brought the number of plots in potatoes above the number which could be successfully harvested in the interval between maturity and the onset of bad weather. More plots would have rendered lifting either impossible or at any rate unsatisfactory from the experimental point of view. Any improvement to be effected had to be accomplished without a large increase in plot number, because of this very practical and relevant restriction. The difficulty was overcome by amalgamating the qualitative and quantitative trials. In 1926, these two totalled 80 plots, a Latin square of 16, and 4 blocks of 16. In 1927, the two investigations were combined in an experiment of 81 plots. In order to do this the quantitative side had to be cut down to three increments of nitrogen and potash, but as will appear later there was a marked improvement of accuracy and a much greater fund of information available from the new design.

4 0 333·5	2 0 379·5	2 P4 382·5	2 0 379·0	4 P2 381·0	0 0 382·0	2 0 380·5	0 P4 335·5	2 M2 389·0
0 M4 308·5	2 S2 421·0	4 M2 430·5	4 P4 396·0	4 0 413·5	2 S4 424·5	2 M4 409·5	4 S4 436·0	0 0 348·5
4 S4 403·0	0 0 356·5	0 P2 365·0	0 S2 401·0	2 M2 420·0	0 M4 364·0	4 0 399·0	4 S2 408·0	0 P2 354·0
2 P2 404·5	0 S2 357·0	4 0 412·5	2 P2 408·5	4 S2 438·5	4 0 428·0	4 S4 412·0	2 S2 411·0	0 M2 361·0
4 M2 440·0	2 P4 323·5	0 S4 362·5	2 P4 403·5	2 0 409·5	0 M2 360·5	0 P4 319·0	2 0 402·5	2 M4 369·5
4 M4 436·5	2 0 394·5	0 0 395·0	4 M4 465·5	0 0 366·5	0 S4 395·5	0 0 349·5	4 0 400·5	4 P2 358·5
0 0 337·5	0 M2 345·0	4 0 440·0	2 0 446·5	2 M4 455·0	4 P4 405·5	0 P4 333·0	2 S4 405·0	4 M2 390·5
0 M4 302·0	2 0 377·0	2 S2 467·5	4 S2 473·0	0 P2 395·5	4 0 411·5	0 0 351·5	0 S2 344·0	4 0 369·0
4 P4 356·5	4 P2 388·0	2 S4 463·5	2 M2 474·0	0 0 411·5	0 S4 401·5	2 P2 400·5	2 0 389·5	4 M4 436·0

Fig. 4. Qualitative and quantitative experiment of 1927.

The two numbers in the upper line represent the quantities of nitrogenous and potassic manures, the kind of the latter used being indicated by S for potassium sulphate in cwt. per acre, M for potassium chloride containing equal amount of potassium, and P for the equivalent low grade salt. The lower numbers represent the yield in lb. of a plot of one-fortieth of an acre.

The quantities of potash and nitrogen are shown below:

Sulphate of ammonia, cwt.	Equivalents of sulphate of potash, cwt. 0	2	4
0	0 0	0 2	0 4
2	2 0	2 2	2 4
4	4 0	4 2	4 4

and the arrangement in Fig. 4 where S, M, P indicate the source of the potash applied.

These nine treatments constituted the block, and of such blocks there were nine in all. The potash had however to be divided out amongst the three kinds, sulphate, muriate and low grade; there being three plots receiving double and three single potash, one of each in each block were allotted to each kind. The manner of allotting these qualitative differences amongst the varying quantities of nitrogen requires detailed description. The actual position of a plot considered only as representing potash and nitrogen interactions was determined entirely by chance. The element of chance also operated largely in the disposition of the qualitative factor, but there was one restriction. The restriction provided that any particular variety of potash manure should occur in the total of the nine blocks in conjunction with every amount of nitrogen three times. In every other way the distribution was at random.

The amount of replication in this experiment varies with each factor or interaction of factors concerned. The number of independent comparisons which can be made is thus summarised:

	Number of comparisons
(1) Action of potash in varying quantites in combination with a standard quantity of nitrogen	27
(2) Action of nitrogen in varying quantites with standard potash (quantitative)	27
(3) Interaction of nitrogen and potash in every combination	9
(4) Between kinds of potash	18
(5) Differential response of kind of potash to quantity of potash	9
(6) Differential response of kind of potash to quantity of nitrogen	9
(7) Differential response of kind of potash to quantity of potash and nitrogen varying simultaneously	3
(8) Elimination of soil heterogeneity	9

The experiments of 1925 and 1926 gave information on sections 1–4, sections 5–7 are additional information and the accuracy of the comparison between kinds of potash is enormously enhanced, there being now 18 comparisons in place of 4.

The advantage of this type of survey experiment is, as has been pointed out, very great. For each comparison an appropriate error is

obtained with respect to which interpretation can be made. Consideration of the mean yields in conjunction with their appropriate errors shows how greatly improved is the standard of accuracy of the qualitative side of the trial. In 1925, although the means showed the apparent order of efficiency to be sulphate, muriate, low grade salts, even by taking the maximum difference sulphate versus low grade salts (a not entirely fair method), the differences were only probably significant and not completely so, and a similar state of affairs is seen in 1926 with respect to the greatest difference, muriate versus low grade salts.

In the 1927 trial, a summary of which is shown in Table VIII, a much closer control is established and for double dressings the depression of the low grade salts is significant. The depressing effect of muriate rests as a probability and the results show that the effect is felt least where the dressings of nitrogen are high—*i.e.* where the manurial effect of potash would be more apparent. The sulphate appears to function normally at all values of nitrogen.

Table VIII. *Analysis of variance*, 1927.

Variance due to	Degrees of freedom	Sums of squares	Mean square
Potash and nitrogen	8	49,905	6,238
Quality of potash	2	14,458	7,229
Quantity *v.* quality of potash	2	1,005	503
Blocks	8	21,442	2,680
Error	60	33,919	565
Total	80	120,729	—

Average yield in tons per acre.

Potash, cwt.	Nitrogen, cwt. 0	2	4	Potash, kind S	M	P
0	6·545	7·061	7·158	6·921	6·921	6·921
2	6·514	7·532	7·357	7·383	7·164	6·858
4	6·193	7·215	7·435	7·348	7·037	6·458

Standard error 0·141 ton.

The analysis of variance shows that differences of decided significance have been obtained both on the quantitative and qualitative questions. The table of average yields shows (i) the responses to increasing dressings of nitrogen, not very large in absolute amount, but capable of fair quantitative estimation in an experiment of the precision actually attained, (ii) a decided response to the first dose of potash, but much less, if any, to the second dose, (iii) that the second dose, when all three kinds of potassic manure are considered together, is deleterious in the absence of nitrogen, but probably becomes beneficial when the total yield is stimulated by heavy nitrogenous dressings.

The table showing the three kinds of potash separately is of special interest in providing unequivocal confirmation of the conclusions indicated, but without sufficient statistical significance, by the earlier experiments; all levels of nitrogenous manuring are here thrown together. With sulphate we have a decided increase from the first dose, and no appreciable decrease due to the second dose. With muriate the yield with double potash is about midway between those obtained with none and with single potash; while with a source of potash which contains much additional sodium chloride, the first dose has on the average no appreciable effect, while the second dose produces a decided loss of yield.

If we contrast the yields at the same level of abundance of potash, we find sulphate beating muriate by 0·22 ton at the single level, and by 0·31 ton at the double level; while it beats the potash manure salts by 0·525 at the single level, and by 0·890 at the double level, the difference being two and one-half to three times as great. It is clear that we must interpret these results, not as due to any difference in availability of the potash, but as due to other effects, presumably the presence of chloride, which effect a quantitative depression of the yield nearly proportional to the quantity of chloride present. The use of no-potash plots designed to show that the crop is really ready to respond to available potash, while essential if availability is in question, are quite superfluous for the examination of effects of this kind, which are most clearly seen with the second dose of potash, to which there is in the present experiment no appreciable response.

Summary.

The development is recorded of the series of experiments with potatoes at Rothamsted during 1925–27, designed to examine the quantitative response of yield to varying quantities of nitrogenous and potassic manures, and to test the relative value with this crop of different sources of potash.

While rather precise comparisons were obtained on the qualitative question by means of Latin squares in 1925–26, the reality of the depression ascribable to chloride could not be demonstrated in these years, but became clearly apparent when in the following year, the qualitative experiment was merged with the quantitative one.

In the earlier quantitative experiments, although satisfactory responses were obtained, the precision of the results left much to be

desired, since only four replicates could be used. When by merging the experiments this was increased to nine replicates, much smaller responses were clearly measurable.

The large and complex type of experiment finally adopted thus supplied more precise information on both heads than could previously be obtained, and in addition to a more thorough exploration of the different combinations possible.

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THE ESTIMATION OF YIELD IN CEREAL CROPS BY SAMPLING METHODS.

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(With Five Text-figures.)

It has long been felt that the use of a reliable sampling method would be highly advantageous for the estimation of yields in experimental work with cereals. Experiments were made during the summer of 1928 with a view to determining the accuracy with which such estimates may be made, and to deciding on a satisfactory sampling technique.

In all cases the number of samples taken from plots whose size varied from about one-seventeenth to one-fifth of an acre, was 30, each sample consisting of the total produce from a metre-length of a single drill. Each sample was tied with string and labelled on the field. Later it was weighed as a whole, after which the heads were cut off and threshed by hand. The grain was then weighed, and the weight corrected for moisture content, so that all calculations were based on dry weight.

Two, and in one case three, sampling methods were tried.

(*a*) The plots, all of which were narrowly rectangular in shape, were divided transversely into three equal parts, and ten samples were taken at random from each part.

(*b*) Six sets, each of which comprised a succession of five contiguous metre-lengths, disposed symmetrically within the plot, were cut as samples, each metre-length being tied separately. A somewhat similar scheme—the "Rod-Row Method,"—has been adopted by American agronomists (1), and it was thought desirable to compare it with a method based on random sampling.

(*c*) Six metre-lengths at equal intervals along the plot were cut from each of five drill-rows chosen at random. This scheme was only tried on one occasion, with wheat. Tables of the primary data will be found in Appendix I.

I. RESULTS WITH BARLEY.

These plots, of areas one-seventeenth to one-eleventh of an acre, and of three different varieties, were each sampled by methods (*a*) and (*b*) above. Total produce and grain were weighed for each metre-length, and results were calculated for straw as well as grain.

The statistical technique known as the "Analysis of Variance" was devised by R. A. Fisher, and first published in its complete form in 1923 (5). The principle of the method is that the total variation between the individual results in a set of data, if measured in terms of the sum of squares of deviations of these results from their general mean, may be analysed into a number of parts by the application of a well-known algebraic identity. This allows of the apportioning of fractions of the total sum of squares to various known causal factors, leaving a residual fraction due to unknown or uncontrolled factors. This latter fraction provides a logical basis for an estimate of the errors of an experiment. Fisher has further shown that the mean value of the fraction ascribable to any factor—the "variance"—is obtained by dividing that fraction by the number of "degrees of freedom" on which it is based, where "degrees of freedom" is used in the sense of "independent comparisons." Thus between n quantities whose mean is fixed there are in general $n - 1$ independent comparisons or degrees of freedom.

In the following pages the experimental results are treated in turn by this method. There are in all cases 29 degrees of freedom, since 30 samples were taken from every plot. In Method (*a*), since each plot was divided into three parts, 10 samples being taken from each part, the total variance may be analysed into a portion representing differences between the mean yield of the parts, and a residue representing differences between metre-lengths within the same part. The former portion may fairly be eliminated as being due to differences in mean fertility between the parts; the latter is used for the estimation of experimental error, representing as it does variance due to smaller differences in fertility within each part, to errors of measurement of the metre-lengths, to loss of grain in threshing, to errors in weighing, etc., etc. In a precisely similar manner the variance of results of Method (*b*) may be divided into two fractions, one due to differences between 5-metre-lengths or "sets," the other due to differences between metre-lengths within the same set. The former would be the basis of the estimate of error were the sets cut and weighed as wholes.

ANALYSES OF VARIANCE OF WEIGHTS PER METRE-LENGTH, IN GRAMS.

1. *Variety* "824."

Method (*a*). *Random sampling.*

1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	2072·50	—	—
Remainder	27	6491·46	240·42	15·51
Total	29	8563·96		

Diminution* of variance = 18·59 per cent.

Standard error of a single metre-length = 15·51 gm.

Hence standard error of mean of 30 = $\frac{15\cdot51}{\sqrt{30}}$ = 2·83 gm.

Mean = 47·29 gm.: hence standard error of mean = 5·99 per cent.

2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	5068·27	—	—
Remainder	27	12331·93	456·74	21·37
Total	29	17400·20		

Diminution of variance = 23·88 per cent.

Standard error of a single metre-length = 21·37 gm.

Hence standard error of mean of 30 = $\frac{21\cdot37}{\sqrt{30}}$ = 3·90 gm.

Mean = 72·97 gm.: hence standard error of mean = 5·35 per cent.

Method (*b*). *Systematic sampling: symmetrical method.*

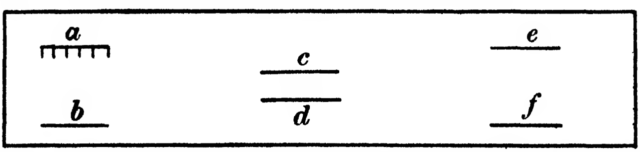

Fig. 1. Plan showing position of "sets" of metre-lengths in Method (*b*).

1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	1247·10	249·42	(15·79)	0·476
Intra-set	24	2350·75	97·95		
Total	29	3597·85	124·06	(11·14)	

Standard error of a single metre-length:

(*a*) as calculated from whole sets = 15·79 gm.;

(*b*) as calculated from individual values = 11·14 gm.

* See p. 230.

Hence standard error of mean:

$$(a) = \frac{15 \cdot 79}{\sqrt{30}} = 2 \cdot 88 \text{ gm.} = 7 \cdot 20 \text{ per cent. of mean;}$$

$$(b) = \frac{11 \cdot 14}{\sqrt{30}} = 2 \cdot 04 \text{ gm.} = 5 \cdot 10 \text{ per cent. of mean.}$$

It is interesting to note that, had the sets been cut as a whole, the standard errors would have been considerably overestimated, owing to the greater variability between than within sets. (Since the arrangement of the metre-lengths was systematic and not random, the standard errors obtained do not provide, in either case, valid estimates of the error of the mean.)

2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	6666·08	1333·22	(36·51)	0·9730
Intra-set	24	4570·83	190·45	—	
Total	29	11236·91	387·48	(19·68)	

Standard error of a single metre-length:

(a) as calculated from whole sets = 36·51 gm.;

(b) as calculated from individual values = 19·68 gm.

Hence standard error of mean:

$$(a) = \frac{36 \cdot 51}{\sqrt{30}} = 6 \cdot 67 \text{ gm.} = 10 \cdot 27 \text{ per cent. of mean;}$$

$$(b) = \frac{19 \cdot 68}{\sqrt{30}} = 3 \cdot 59 \text{ gm.} = 5 \cdot 54 \text{ per cent. of mean.}$$

As before there is a much higher variation between than within sets. The significance of this difference is easily found by R. A. Fisher's "z" test. "z" is half the difference between the natural logarithms of the two variances, and its standard error depends only on the number of degrees of freedom on which the variances are based. Tables have been provided (4) showing the value of "z" which must be attained for two different levels of significance, the 5 per cent. and the 1 per cent. points. If the 5 per cent. point of "z" is reached, it is to be understood that as great a difference between the two variances as was actually observed, would only occur by chance, from homogeneous material, once in 20 samples. Taking the 5 per cent. point, then, as a convenient minimum level for significance, the difference here found is hardly significant in the case of the grain, but highly significant with straw. The 5 per cent. point of "z" is 0·4817, and the 1 per cent. point, 0·6799.

2. *Variety "Spratt Archer."*

Method (*a*). *Random sampling.*

1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	1195·27	—	—
Remainder	27	6778·17	251·04	15·84
Total	29	7973·44		

Diminution of variance = 9·70 per cent.

Standard error of a single metre-length = 15·84 gm.

Hence standard error of mean $= \frac{15{\cdot}84}{\sqrt{30}} = 2{\cdot}89$ gm. = 5·72 per cent. of mean.

2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	2871·63	—	—
Remainder	27	19112·13	707·86	26·61
Total	29	21983·76		

Diminution of variance = 6·62 per cent.

Standard error of a single metre-length = 26·61 gm.

Hence standard error of mean $= \frac{26{\cdot}61}{\sqrt{30}} = 4{\cdot}86$ gm. = 6·09 per cent. of mean.

Method (*b*). *Systematic sampling: symmetrical method.*

1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	1568·31	313·66	(17·71)	0·2914
Intra-set	24	4203·37	175·14	—	
Total	29	5771·68	199·02	(14·11)	

Standard error of a single metre-length:

(*a*) as calculated from whole sets = 17·71 gm.;

(*b*) as calculated from individual values = 14·11 gm.

Hence standard error of mean:

$$(a)\ = \frac{17{\cdot}71}{\sqrt{30}} = 3{\cdot}23 \text{ gm.} = 6{\cdot}57 \text{ per cent. of mean;}$$

$$(b)\ = \frac{14{\cdot}11}{\sqrt{30}} = 2{\cdot}58 \text{ gm.} = 5{\cdot}24 \text{ per cent. of mean.}$$

2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	2852·58	570·51	(23·89)	0·2773
Intra-set	24	7864·37	327·68	—	
Total	29	10716·95	369·55	(19·22)	

Standard error of a single metre-length:

(*a*) as calculated from whole sets = 23·89 gm.;

(*b*) as calculated from individual values = 19·22 gm.

Hence standard error of mean:

$$(a) \quad = \frac{23{\cdot}89}{\sqrt{30}} = 4{\cdot}36 \text{ gm.} = 6{\cdot}95 \text{ per cent. of mean;}$$

$$(b) \quad = \frac{19{\cdot}22}{\sqrt{30}} = 3{\cdot}51 \text{ gm.} = 5{\cdot}59 \text{ per cent. of mean.}$$

Here, although the inter-set is greater than the intra-set variance, the difference is not great, and falls short of significance when tested by the "z" method.

3. *Variety "Plumage Archer."*

Method (*a*). *Random sampling.*

1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	2870·52	—	—
Remainder	27	9319·32	345·16	18·58
Total	29	12189·84		

Diminution of variance = 17·89 per cent.

Standard error of a single metre-length = 18·58 gm.

Hence standard error of mean $= \frac{18{\cdot}58}{\sqrt{30}} = 3{\cdot}39$ gm. = 7·47 per cent. of mean.

2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	13502·00	—	—
Remainder	27	24293·65	899·76	30·00
Total	29	37795·65		

Diminution of variance = 30·96 per cent.

Standard error of a single metre-length = 30·00 gm.

Hence standard error of mean $= \frac{30{\cdot}00}{\sqrt{30}} = 5{\cdot}477$ gm. = 8·31 per cent. of mean.

Method (b). Systematic sampling: symmetrical method.

1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	5733·95	1146·79	(33·86)	0·9842
Intra-set	24	3845·30	160·22	—	
Total	29	9579·25	330·32	(18·17)	

Standard error of a single metre-length:

(*a*) as calculated from whole sets = 33·86 gm.;

(*b*) as calculated from individual values = 18·17 gm.

Hence standard error of mean:

$$(a) = \frac{33{\cdot}86}{\sqrt{30}} = 6{\cdot}18 \text{ gm.} = 15{\cdot}77 \text{ per cent. of mean;}$$

$$(b) = \frac{18{\cdot}17}{\sqrt{30}} = 3{\cdot}32 \text{ gm.} = 8{\cdot}46 \text{ per cent. of mean.}$$

2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	7924·23	1584·85	(39·81)	0·8012
Intra-set	24	7661·56	319·23	—	
Total	29	15585·79	537·44	(23·18)	

Standard error of a single metre-length:

(*a*) as calculated from whole sets = 39·81 gm.;

(*b*) as calculated from individual values = 23·18 gm.

Hence standard error of mean:

$$(a) = \frac{39{\cdot}81}{\sqrt{30}} = 7{\cdot}27 \text{ gm.} = 13{\cdot}78 \text{ per cent. of mean;}$$

$$(b) = \frac{23{\cdot}18}{\sqrt{30}} = 4{\cdot}23 \text{ gm.} = 8{\cdot}03 \text{ per cent. of mean.}$$

The 5 per cent. of "z" is 0·4817, and the 1 per cent. point, 0·6799. The significance of the difference between the intra- and inter-set variance therefore exceeds 1 in 100 both for grain and for straw. The effect of this is seen in the very much higher estimate of standard error obtained from whole sets as compared with individual metre-lengths.

II. RESULTS WITH WHEAT.

Variety "Red Standard."

Method (*a*). *Random sampling.*

1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	1562·55	—	—
Remainder	27	8476·48	313·94	17·72
Total	29	10039·03		

Diminution of variance = 9·31 per cent.

Standard error of a single metre-length = 17·72 gm.

Hence standard error of mean $= \frac{17 \cdot 72}{\sqrt{30}} = 3 \cdot 24$ gm. = 8·08 per cent. of mean.

2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	1122·02	—	—
Remainder	27	74295·45	2751·68	52·46
Total	29	85524·47		

Diminution of variance = 6·69 per cent.

Standard error of a single metre-length = 52·46 gm.

Hence standard error of mean $= \frac{52 \cdot 46}{\sqrt{30}} = 9 \cdot 58$ gm. = 8·72 per cent. of mean.

Method (*b*). *Systematic sampling: symmetrical method.*

1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	1469·41	293·88	(17·14)	0·4044
Intra-set	24	3141·50	130·90	—	
Total	29	4610·91	159·00	(12·61)	

Standard error of a single metre-length:

(*a*) as calculated from whole sets = 17·14 gm.;

(*b*) as calculated from individual values as previous = 12·61 gm.

Hence standard error of mean:

$$(a) = \frac{17 \cdot 14}{\sqrt{30}} = 3 \cdot 13 \text{ gm.} = 8 \cdot 09 \text{ per cent. of mean;}$$

$$(b) = \frac{12 \cdot 61}{\sqrt{30}} = 2 \cdot 31 \text{ gm.} = 5 \cdot 96 \text{ per cent. of mean.}$$

2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	10036·45	2007·29	(44·80)	0·4732
Intra-set	24	18699·00	779·13	—	
Total	29	28735·45	990·88	(31·48)	

Standard error of a single metre-length:

(*a*) as calculated from whole sets = 44·80 gm.;

(*b*) as calculated from individual metres = 31·48 gm.

Hence standard error of mean:

$$(a) = \frac{44\cdot80}{\sqrt{30}} = 8\cdot18 \text{ gm.} = 9\cdot04 \text{ per cent. of mean;}$$

$$(b) = \frac{31\cdot48}{\sqrt{30}} = 5\cdot75 \text{ gm.} = 6\cdot35 \text{ per cent. of mean.}$$

In this case the differences between inter- and intra-set variances just fail to reach the 1 in 20 level of significance ("z" = 0·4817) for straw, and is smaller for grain. There is therefore little difference between standard errors based on the two variances.

Method (c). Systematic sampling: Random Row method.

1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-row	4	21922·83	5480·71	(74·03)	1·4109
Intra-row	25	7154·37	286·17	—	
Total	29	29077·20	1002·66	(31·66)	

Standard error of a single metre-length:

(*a*) as calculated from whole rows = 74·03 gm.;

(*b*) as calculated from individual metres = 31·66 gm.

Hence standard error of mean, as calculated from individual metre-lengths $= \frac{31\cdot66}{\sqrt{30}} = 5\cdot78$ gm. = 11·33 per cent. of mean.

2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-row	4	92529·83	23132·46	—	1·4250
Intra-row	25	33452·49	1338·10	—	
Total	29	125982·32	4344·22	(65·91)	

Standard error of a single metre-length = 65·91 gm.

Hence standard error of mean $= \frac{65\cdot91}{\sqrt{30}} = 12\cdot03$ gm. = 9·67 per cent. of mean.

As the very high values of "z" indicate, the variation between rows has been very much greater than within rows. This was largely due to the fact that an edge-row was sampled, and exaggerates somewhat the danger of systematic sampling of this type. Partial choking of drill-coulters, nearness of rows to field-drains, and many other factors, do, however, tend to make rows as a whole differ widely from their neighbours, and add weight to the case for random sampling.

RÉSUMÉ OF RESULTS.

The most important result that emerges is that with plots having an area of about one-sixteenth of an acre, a "random sampling" method will provide an estimate of yield with a standard error of less than 6 per cent. when 30 samples of metre-length of drill are taken. This would indicate that with plots one-fortieth of an acre in area, the average standard error should be not more than 5 per cent. It is customary at Rothamsted to have experimental plots of about this area, and since the standard error of such plots arising from causes other than sampling errors has been shown to be about 8–10 per cent., the additional inaccuracy introduced by the use of the sampling method described, will be quite small. Thus a standard error of 8 per cent. is increased to 9·4 per cent., and one of 10 per cent. only to 11·2 per cent. by the superposition of a further error of 5 per cent.

As the figures for "Percentage of variance eliminated" show very clearly, it is of great advantage to divide the area to be sampled into a small number of parts within each of which an equal number of samples is taken. By this means, and by the use of R. A. Fisher's statistical technique, the Analysis of Variance, a substantial reduction in the standard error may be effected.

Certain disadvantages of the systematic methods tried also stand out clearly. In the first place, the "rod-row" method used extensively in America is shown to suffer from the grave defect that the unit is often too coarse. This will be referred to later. Secondly, any attempt to reduce the labour of sampling by taking samples only from a small number of rows, whether these be chosen systematically or at random, is liable to lead to an increased estimate of error owing to the difference between rows as wholes—*i.e.* to the greater variation between than within rows. On the one occasion on which the method was adopted, the intra-row correlation, easily calculated from the ratio of intra-row and total variance (see R. A. Fisher (4) p. 191), is $+0·6635$, and highly

As before yields were calculated not only from the 10 samples from each plot, but also from 9, 8, 5, 4 from the centre, and 4 from the ends. Analyses were made of figures for total yields, for estimates from 10 samples, and from 5 samples. Series II, III and IV, were grouped for this purpose.

4. Analysis of figures for toal yields.

Fraction	Degrees of freedom	Sum of squares	Mean square	½ log mean square	Standard error	Standard error %
Sets	17	2293·73	134·925	—	—	—
Remainder	36	119·38	3·316	0·5994	1·821	6·62
Total	53	2413·11				

5. Analysis of figures for ten square-yard samples.

Fraction	Degrees of freedom	Sum of squares	Mean square	½ log mean square	Standard error	Standard error %
Sets	17	1116·93	65·702	—	—	—
Remainder	36	186·75	5·188	0·8232	2·278	9·80
Total	53	1303·68				

6. Analysis of figures for five square-yard samples.

Fraction	Degrees of freedom	Sum of squares	Mean square	½ log mean square	Standard error	Standard error %
Sets	17	1046·06	—	—	—	—
Remainder	36	275·30	7·647	1·0172	2·765	12·01
Total	53	1321·36				

The additional standard error per plot due to sampling may now be estimated for the values of the mean squares given above:

9 rod-rows	3·67 per cent.
4 ,, ...	5·32 ,,
10 square yards	7·23 ,,
5 ,, ...	10·03 ,,

The first three of these standard errors are fairly small, and, since a rod is roughly equivalent to 5 metres, of about the same order as those obtained in the random sampling method. It must be noted, however, that these estimates are subject to very large sampling errors, owing to the fact that they are calculated from the differences between variances.

The standard error of the difference between two variances based on N degrees of freedom is given by the formula:

$$s_d = \sqrt{\frac{2}{N}(\sigma_1^4 + \sigma_2^4 - 2r_{\sigma_1^2\,\sigma_2^2}\,\sigma_1^2\,\sigma_2^2)},$$

where σ_1^2, σ_2^2 are the two variances, and $r_{\sigma_1^2\,\sigma_2^2}$ is the correlation between them in samples.

Now it has recently been shown by Wishart ((6) p. 43), that if $\rho_{1.2}$ is the correlation between the two variates in the original population $r_{\sigma_1^2\,\sigma_2^2} = \rho^2_{1.2}$, exactly.

Then, substituting for the population parameters $\sigma_1^2, \sigma_2^2, \rho_{1.2}$, the observed values $s_1^2, s_2^2, r_{1.2}$, which are the best available estimates, we have:

$$s_d = \sqrt{\frac{2}{N}\left(s_1^4 + s_2^4 - 2r^2_{1.2}s_1^2s_2^2\right)}$$

is in each case the residual correlation between the estimates of yield obtained by the two methods—*i.e.* the correlation calculated from the "Remainder" variances and covariances[1]. The covariances must therefore be analysed in precisely the same manner as the variances, before the correlation coefficients can be obtained.

(*a*) *Total yields and* 9 *rod-rows.*

Fraction	Covariance	Correlation coefficient
Sets	150·733	+0·5960
Remainder	89·056	+0·6464
Total	239·789	+0·6076

(*b*) *Total yields and* 4 *rod-rows.*

Fraction	Covariance	Correlation coefficient
Sets	154·669	+0·7061
Remainder	105·855	+0·6989
Total	260·524	+0·7030

) *Total yields and* 10 *square yards.*

Fraction	Covariance	Correlation coefficient
Sets	1528·066	+0·9547
Remainder	88·286	+0·5913
Total	1616·352	+0·9113

(*d*) *Total yields and* 5 *square yards.*

Fraction	Covariance	Correlation coefficient
Sets	1451·438	+0·9370
Remainder	106·399	+0·5869
Total	1557·837	+0·8724

Using the underlined correlation coefficients, the following results are obtained for the accuracy of the difference between the residual variances:

(*a*) $1{\cdot}932 \pm 1{\cdot}878$,

(*b*) $3{\cdot}324 \pm 2{\cdot}077$,

(*c*) $1{\cdot}872 \pm 1{\cdot}199$,

(*d*) $4{\cdot}331 \pm 1{\cdot}699$.

Hence only the last can be said to differ significantly from zero, and in no case is the value of the sampling error established with any approach to certainty.

The same result may be arrived at more simply by the use of R. A. Fisher's "*z*" transformation, by means of which the significance of a difference between variances may be tested directly.

[1] The "covariance" is the average value of the sum of products of deviations from the mean, and is obtained by dividing that quantity by the appropriate number of degrees of freedom.

1. Arny and Garber's data.

	Total yield and 9 rod-rows	Total yield and 4 rod-rows	5 % point of "z"	1 % point of "z"
"z"	0·1676	0·2606	0·3425	0·4890

2. Arny and Steinmetz' data.

	Total yield and 10 square yards	Total yield and 5 square yards	5 % point of "z"	1 % point of "z"
"z"	0·2238	0·4178	0·2596	0·3702

Here again only the last result attains even the 5 per cent. point of "z"—*i.e.* only in the last case would such a difference between variances occur as infrequently as once in 20 samples from a homogeneous population. This confirms the conclusion that little reliance can be placed on the calculated values of the errors due to sampling, and illustrates in a striking manner one of the great disadvantages of a systematic as compared with a random sample. A random sample gives a direct estimate of the errors due to sampling, an estimate, therefore, of far greater accuracy than the indirect estimate obtained as above. It is of great importance that such an estimate should be arrived at, since the improvement of experimental technique depends on a knowledge of the causes of inaccuracy. Data such as those of Arny and Garber do not distinguish adequately between errors due to insufficient replication, and errors of sampling within the plots. If the main sources of error were the former, the taking of a greater number of samples from each plot would do little towards increasing the accuracy of the experiment, and vice versa.

The only manner in which direct estimates of the sampling error of a systematic method can be obtained, is by making a series of observations in which at least duplicate sets of samples are taken from each plot. These sets must further be such that they form a random sample from the whole population of possible sets. Only under these circumstances can a valid estimate of error be made. Hence there is the initial condition that such a population exists, for if it does not exist, no random sample can be made from it, nor can a standard error be calculated. In the present instance it is very difficult to see how such populations can be constructed. It is, however, possible, to devise systematic methods which do admit of the calculation of a valid standard error. The method used by Engledow (3), is a case in point. Here, in one variant of the method, 1-ft. samples are cut as in Fig. 4.

AB represents the width of the area to be sampled and is measured parallel with the drill-rows. 1, 2, 3 ... represent the 10th, 20th, 30th ...

drill rows. Samples are taken successively from these rows, there being a constant lateral shift from sample to sample. When a complete traverse of the area has been effected, a fresh start is made from the far side, as on the 70th drill-row in the figure.

If n samples are taken in passing from side to side of the area, there may be considered to be n possible starting-points along the base line AB. It would then be possible to get a valid estimate of error by taking

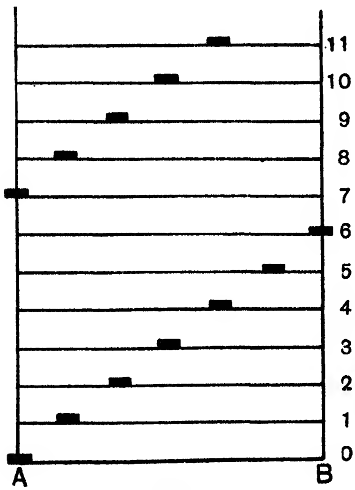

Fig. 4.

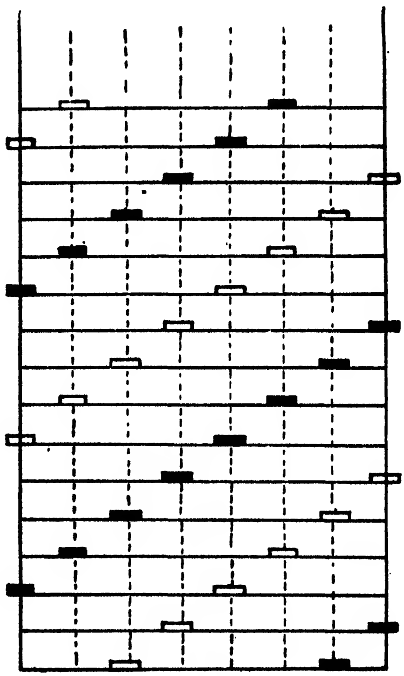

Fig. 5.

duplicate sets from each area to be sampled, the starting-points of the sets being chosen at random from among the n possible points. Thus in one of the areas, the samples might be taken as in Fig. 5.

A systematic arrangement, such as that of Engledow, samples the area very effectively, but it can scarcely be maintained that this advantage outweighs the disadvantage that the samples do not in themselves yield an estimate of their standard error, as would be the case with a random sample. Thus it should be noted that n separate *sets* of systematic samples would be required to yield the same information as to sampling errors as a single set of n samples distributed at random.

This is true whatever the number of samples in the systematically arranged set. It can be ensured moreover that the random method does sample the whole area, by dividing the area into a number of subdivisions. Then if the same number of samples is taken from each part of the area, there is secured both an effective distribution of samples, and an arrangement which permits of the elimination of that portion of the total variance which is due to differences in the mean fertility of the subdivisions of the area. This is, of course, equally possible with a systematic method, but although the necessary statistical technique—the Analysis of Variance—has been available for some years, no such use has previously been made of it.

The advantage to be gained by the subdivision of the area to be sampled has already been referred to. It may be useful, however, to collect the figures which bear on the point. Below are given the percentages of the total variance which are eliminated as being due to difference in soil fertility over subdivisions of the areas sampled, and which, consequently, represent percentage reductions of the variance on which the standard errors are based.

		% reduction in variance	
Crop	Variety	Grain	Straw
Barley	"824"	18·59	23·88
,,	"Spratt Archer"	9·70	6·62
,,	"Plumage Archer"	17·89	30·96
Wheat	"Red Standard"	9·31	6·69

It will be seen that in no case is less than 6·5 per cent. of the variance removed by this procedure, the mean reduction being 15·46 per cent.

With regard to the size and nature of the sampling unit, our results show conclusively that the rod-row is too coarse a unit. The significant intra-class correlations obtained when separate weighings are made of the five metre-lengths in each sampling unit of method (*b*), are as shown below:

		Intra-class correlations (where significant)	
Crop	Variety	Grain	Straw
Barley	"824"	—	+0·7966
,,	"Spratt Archer"	—	—
,,	"Plumage Archer"	+0·6886	+0·3856
Wheat	"Red Standard"	—	+0·1866*

* p = about 0·053.

Except where otherwise stated the level of significance has been taken as $p = 0{\cdot}050$, where p is the probability that so high a value could be obtained by chance.

These figures indicate that there has been a considerable loss of information over that provided by the same number of metre-lengths arranged at random over the area. In certain other investigations by the author it has even appeared that the metre may be too long, significant correlations having been obtained between successive half-metre-lengths of drill. In view of this experiments were tried with a dissected 4-ft.-length, each foot being separated from its neighbour by 2 ft. of unsampled corn. No significant intra-class correlations were obtained with this method, even when "neighbouring" foot-lengths were compared. Engledow uses the foot-length as his unit, and points out that smaller lengths would be impracticable owing to the increased importance of end-errors. There being no intra-class correlation between the parts of a dissected 4-ft.-length, it is better to use 30 of such units rather than 120 separately located 1-ft.-lengths, since the location of each of the former units fixes 4 ft. at once, thus reducing the labour involved in sampling.

What has been said of using the rod-row as a unit will be equally true of the square yard. In fact it seems highly probable that the loss of information would be even greater in the case of five metres lying side by side than if they were end to end.

In conclusion, I wish to thank Dr R. A. Fisher of this Station for valuable criticism and advice; and Messrs H. J. Johnson and T. W. Simpson of Armstrong College, for carrying out almost the whole of the experimental work.

SUMMARY.

1. Cereal plots were sampled by three different methods; two systematic, and one involving a random location of sampling units.

2. The disadvantages of the systematic methods as compared with random sampling, emerged clearly.

3. These disadvantages were further emphasised in an analysis of earlier data on sampling methods. For this purpose the methods and results of certain recent contributions to statistical theory were used.

4. By the use of a random sampling method, the variance due to sampling errors may be made a satisfactorily small fraction of the total variance of cereal plots one-fortieth of an acre in area.

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APPENDIX I.

Tables of primary data.

(The figures are in grams per metre: the grain figures in grams of dry weight per metre.)

I. BARLEY.

1. *Variety* "824."

Method (*a*). *Random sampling.*

Block A		Block B		Block C	
Grain	Straw	Grain	Straw	Grain	Straw
28·47	61·3	56·18	90·5	22·56	34·3
78·83	101·7	64·04	95·2	41·99	62·3
93·87	137·9	45·96	62·6	14·45	37·9
35·40	58·1	55·17	88·7	28·90	50·8
53·31	51·9	69·62	127·6	33·04	50·9
55·34	73·5	36·08	79·3	27·88	63·0
65·99	35·9	54·07	84·0	41·99	53·3
44·61	72·2	35·74	78·7	49·09	81·9
61·42	86·3	47·82	92·4	45·37	63·3
29·40	50·2	50·53	90·2	51·45	73·1
546·64	729·0	515·21	889·2	356·72	570·8

Grand total		Grand mean	
Grain	Straw	Grain	Straw
1418·57	2189·0	47·286	72·967

Method (*b*). *Systematic sampling: symmetrical method.*

Set *a*		Set *b*		Set *c*		Set *d*		Set *e*		Set *f*	
Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
13·10	23·5	22·73	37·1	35·82	60·6	41·15	63·3	43·85	45·1	37·68	62·4
34·81	45·8	34·98	51·6	47·91	86·3	53·40	85·8	52·89	82·4	28·98	50·7
36·67	59·6	28·22	39·6	27·97	67·9	38·61	68·3	58·38	94·9	40·89	69·6
44·36	34·1	46·89	61·5	44·19	77·7	57·96	92·4	47·23	80·1	44·35	68·5
31·78	58·5	24·76	37·7	58·21	88·1	49·51	93·4	29·57	85·0	43·34	75·7
160·72	221·5	157·58	227·5	214·10	380·6	240·63	403·2	231·92	387·5	195·24	326·9

Grand total		Grand mean	
Grain	Straw	Grain	Straw
1200·19	1947·2	40·006	64·907

2. *Variety "Spratt Archer."*

Method (*a*). *Random sampling.*

Block A		Block B		Block C	
Grain	Straw	Grain	Straw	Grain	Straw
63·84	122·4	80·94	117·8	62·01	86·7
62·40	108·2	47·12	70·5	43·41	65·2
34·06	69·0	78·57	109·8	42·05	61·9
41·73	80·3	37·30	39·9	51·40	73·1
35·24	64·5	61·38	82·5	35·16	55·6
48·95	79·2	68·67	109·3	51·40	68·1
30·49	61·5	79·75	119·3	34·14	54·9
85·30	162·3	57·50	64·4	57·66	81·2
48·55	69·7	34·45	95·5	36·51	50·9
23·68	65·1	48·39	40·9	35·80	62·8
474·24	882·2	594·07	849·9	449·54	660·4

Grand total		Grand mean	
Grain	Straw	Grain	Straw
1517·85	2392·5	50·595	79·75

Method (*b*). *Systematic sampling: symmetrical arrangement.*

Set *a*		Set *b*		Set *c*		Set *d*		Set *e*		Set *f*	
Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
63·87	74·4	27·80	27·1	51·71	68·8	52·97	85·3	59·14	70·0	48·24	59·9
19·86	25·5	47·90	51·3	53·82	63·3	40·89	56·6	46·55	55·9	36·16	64·2
34·81	45·8	29·82	39·7	60·66	66·2	48·58	57·5	57·96	85·4	83·39	99·3
44·10	102·8	47·48	51·8	45·79	58·8	62·61	49·9	63·11	77·3	49·93	61·9
32·95	49·0	53·23	62·0	29·99	34·5	53·74	71·4	52·97	71·3	77·31	96·5
195·59	297·5	206·23	231·9	241·97	291·6	258·79	320·7	279·73	359·9	295·03	381·8

Grand total		Grand mean	
Grain	Straw	Grain	Straw
1477·34	1883·4	49·245	62·780

3. *Variety "Plumage Archer."*

Method (*a*). *Random sampling.*

Block A		Block B		Block C	
Grain	Straw	Grain	Straw	Grain	Straw
21·15	34·3	75·70	109·4	45·62	56·0
49·34	77·7	37·94	48·1	32·28	31·8
74·53	118·9	49·93	61·9	8·79	11·6
76·03	124·0	56·10	67·6	22·56	29·3
93·77	167·6	62·35	87·2	23·83	33·8
54·33	90·4	67·25	91·4	16·14	22·9
21·62	28·7	62·10	86·5	36·67	40·6
65·97	119·7	31·35	48·9	48·07	60·1
43·87	85·6	41·57	56·8	42·08	48·2
23·21	53·7	38·36	35·6	39·71	50·0
523·82	900·6	522·65	693·4	315·75	384·3

Grand total		Grand mean	
Grain	Straw	Grain	Straw
1362·22	1978·3	45·407	65·943

Method (*b*). *Systematic sampling: symmetrical method.*

Set *a*		Set *b*		Set *c*		Set *d*		Set *e*		Set *f*	
Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
45·79	53·8	37·26	37·9	41·15	50·3	33·46	56·4	43·77	58·2	28·30	55·5
22·98	37·8	22·64	38·2	71·99	90·8	34·98	32·6	56·61	62·0	24·25	50·3
31·77	35·4	29·32	42·3	75·87	102·2	51·79	71·7	36·67	55·6	14·28	20·1
27·54	38·4	30·75	44·6	83·90	112·7	67·59	91·0	32·53	44·5	6·17	9·7
22·31	22·6	34·39	44·3	42·58	53·6	37·60	53·5	58·04	71·3	29·66	44·9
150·39	188·0	154·36	207·3	315·49	409·6	225·42	305·2	227·62	291·6	102·66	180·5

Grand total		Grand mean	
Grain	Straw	Grain	Straw
1175·94	1582·2	39·198	52·740

II. WHEAT.

Variety "Red Standard."

Method (*a*). *Random sampling.*

Block A		Block B		Block C	
Grain	Straw	Grain	Straw	Grain	Straw
26·50	66·1	36·98	107·7	47·85	154·8
37·14	91·5	37·29	111·3	120·10	344·4
30·49	74·0	42·14	139·1	51·06	147·7
37·14	105·5	34·56	87·8	45·74	125·5
52·39	129·0	35·11	88·1	47·23	110·6
36·04	93·9	41·44	110·0	17·98	62·0
48·01	181·6	37·37	73·2	24·39	64·8
20·33	61·0	46·52	109·5	58·95	143·6
12·12	54·5	25·33	65·6	36·83	103·9
28·07	102·1	37·22	73·4	48·87	115·5
328·23	959·2	373·96	965·7	499·00	1372·8

Grand total		Grand mean	
Grain	Straw	Grain	Straw
1201·19	3297·7	40·040	109·923

Method (*b*). *Systematic sampling: symmetrical method.*

Set *a*		Set *b*		Set *c*		Set *d*		Set *e*		Set *f*	
Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
41·99	88·3	27·52	60·8	38·70	76·5	20·09	47·3	47·38	112·4	29·40	79·4
23·30	51·2	40·66	85·0	46·29	96·8	34·25	79·2	41·28	114·2	45·43	76·9
42·22	92·0	31·67	67·5	30·49	74·0	41·12	100·4	32·37	88·6	34·18	87·3
26·89	65·6	35·18	79·0	60·52	137·6	42·30	94·9	46·60	111·4	36·20	93·7
40·50	83·2	30·81	68·6	88·75	217·5	29·40	66·4	41·83	123·5	33·70	93·9
174·90	380·3	165·84	360·9	264·75	602·4	167·16	388·2	209·46	550·1	178·91	431·2

Grand total		Grand mean	
Grain	Straw	Grain	Straw
1161·02	2713·1	38·701	90·473

Method (*c*). *Random row method.*

Row 1		Row 3		Row 19		Row 20		Row 24	
Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
94·58	184·9	22·08	60·3	30·49	69·5	32·13	163·6	9·53	25·9
90·02	221·2	33·93	80·5	36·59	104·4	29·03	68·2	36·34	96·7
86·41	195·4	33·93	67·5	30·24	61·8	48·88	94·1	42·18	94·9
114·93	267·2	30·24	73·8	50·42	93·3	24·91	110·0	51·02	98·6
148·95	311·6	46·99	121·3	46·39	165·0	51·20	127·4	60·99	136·0
93·55	221·1	30·07	76·0	50·59	133·1	30·07	96·0	43·29	112·6
628·44	1401·4	197·24	479·4	244·72	627·1	216·22	659·3	243·35	564·7

Grand total		Grand mean	
Grain	Straw	Grain	Straw
1529·77	3731·9	50·992	124·397

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NUTRITIVE VALUE OF PASTURE.

IV. THE INFLUENCE OF THE INTENSITY OF GRAZING ON THE YIELD, COMPOSITION AND NUTRITIVE VALUE OF PASTURE HERBAGE (PART II).

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Introduction.

The main object of this series of pasture investigations is to secure comprehensive information concerning the composition, digestibility and nutritive value of pasture grass in its various stages of growth. The results of the first two investigations, carried out during the seasons of 1925 and 1926 respectively (1, 2), demonstrated that, under a system of weekly cutting, a pasture will yield, irrespective of its botanical composition, a herbage whose dry matter possesses the character of a protein concentrate of high digestibility and nutritive value. Further, under such a system of treatment, this protein concentrated character is retained substantially throughout the entire season, particularly when rainfall distribution, soil conditions and botanical composition combine to keep up the level of productivity during the summer months. A third investigation (3), carried out during 1927, led to the conclusion that the differences in chemical composition, both organic and inorganic, between pasture grass cut at weekly and fortnightly intervals are inconsiderable. The dry matter of pasture herbage grown under a system of fortnightly cutting is a protein concentrate equal in digestibility and nutritive value to that obtained by weekly cutting. At the end of a fortnight, the herbage still consists of the same immature, non-lignified tissue as it did at the end of a week's growth. Moreover, by systematic cutting at fortnightly intervals, these characteristics are retained over the entire season.

The investigation to be described in the present communication was designed to ascertain the effects of lengthening the interval between successive cuttings to three weeks on the yield, composition and nutritive value of pasture herbage. By adopting, year by year in a

systematic manner, a more and more lenient system of cutting, it is hoped ultimately to be able to give a complete answer to the following questions:

(1) At what stage in the growth of pasture grass does lignification, with consequent lowering of digestibility, set in?

(2) Is there a stage of growth, prior to lignification, when the food material in pasture grass is so balanced, in respect of digestible protein and non-protein constituents, as to render it more adapted to the requirements of grazing animals than is the case with grass grown under systems of weekly and fortnightly cuts? In its early stages of growth, the dry matter of pasture grass is a protein concentrate with a nutritive ratio comparable in narrowness with that of linseed cake (1, 2, 3). Such young pasturage is therefore not designed, when it forms the sole constituent of the diet, to produce the best possible results in growing, milking and fattening animals. For this reason, it is important to find out how the nutritive ratio of the grass widens as the interval between successive cuttings is lengthened. The results of such inquiry should be capable of profitable application in rotational grazing practice.

(3) Under what system of cutting, or rotational grazing, does a pasture yield the maximum amount of starch equivalent in a season? An answer to this question will be significant in connection both with the practice of rotational grazing and with the proposals which have been put forward for cutting and conserving pasture grass in the artificially-dried form or in the form of silage (1, 2, 3). It has already been demonstrated (3) that although intensification of the system of cutting tends to depress productivity, the difference between weekly and fortnightly systems is not sufficient to bring out this effect very prominently. When meteorological conditions are favourable to active growth of herbage, productivity under the two systems of cutting does not differ very greatly, certainly by not more than 10 per cent. in favour of the more lenient system. On the other hand, however, when meteorological conditions lead to a slowing-up in the rate of growth on pastures, as during a spell of droughty weather, productivity under a system of cutting at fortnightly intervals becomes markedly higher than under a system of weekly cutting. The question of the maximum yield of digestible food from a pasture is bound up with the investigation of the process of lignification in the herbage, since it is reasonable to assume that the conditions for such maximum yield will be realised when the interval between successive cuttings, or grazings, is as long as possible. The length of this optimum interval will naturally be conditioned by the

time required by the young shoots of grass to reach the stage of growth at which lignification sets in.

GENERAL ARRANGEMENT OF INVESTIGATION.

The present trial was carried out during the season of 1928 on the light-land pasture on which the 1925 (weekly cutting)(1) and the 1927 (fortnightly cutting)(3) experiments had been conducted. With the exception of a dressing of 10 cwt. of basic slag to the acre, which was applied in the autumn following the conclusion of the 1925 investigation, this pasture has not received any manurial treatment during the last decade.

The pasture plot, which comprised 1·2 acres, was divided into 24 equal sub-plots, each measuring 210 by 23·81 links. Sub-plots 8 and 9 were reserved for cutting at weekly and fortnightly intervals respectively, so that in this way a direct comparison could be obtained of the yields of dry matter obtained under weekly, fortnightly and 3-weekly systems of cutting. The herbage on sub-plot 24 was permitted to grow unchecked for the purpose of hay and aftermath trials.

The main experiment was carried out on the remaining 21 sub-plots. A sub-plot was cut every day by means of a motor lawn mower, so that the whole pasture plot was cut over once in every three weeks. After cutting, the produce, which included every form of herbage in the sub-plots, was weighed, mixed thoroughly and sampled for chemical analysis. A suitable bulk was then transported to the School of Agriculture for feeding to sheep on which digestion experiments were being carried out. The conditions of this trial, therefore, were similar to those which would obtain under a system of rotational grazing, where the interval between successive grazings was of three weeks' duration, and where the enclosures, during the grazing periods, were so stocked as to ensure close and uniform cropping of the herbage.

During the winter preceding the experiment, the grass on the plot had been held in check by being grazed at intervals by the sheep flock. The plot was lightly rolled in the early days of March, and on the 13th, 14th and 15th days of this month, the lawn mower was taken over the entire area. At this date, the plot had a fresh verdant appearance, and its content of old, rough herbage was very small. This was attributable to the influence of the systematic cutting which had been carried out during the previous season. Systematic cutting of the daily sub-plots was commenced on March 22. By April 11, therefore, the whole plot had been cut over once, and from that date onward, the herbage obtained

by the successive daily cutting of the sub-plots represented three weeks old grass.

For reasons cited in a previous communication (3), it has been deemed advisable to withhold the detailed discussion of the seasonal productivity of the pasture in relation to meteorological conditions and other influencing factors until the completion of this series of investigations. In this paper, only those yield and meteorological data will be brought forward which have a direct bearing on the nutritional aspects of the investigation. In respect of weather, it will be sufficient to summarise the monthly records during the season of the experiment.

Table I. *General meteorological conditions during the grazing season of* 1928.

Month	Apr.	May	June	July	Aug.	Sept.
Rainfall in in.	0·84	2·49	2·26	2·17	1·80	0·59
Average per day	0·028	0·080	0·075	0·070	0·060	0·020
No. of rainy days (0·01 in. or over)	12	15	15	8	13	4
Sunshine in hours	109	164	197	280	203	210
Average per day	3·6	5·3	6·6	9·0	6·6	7·0
No. of sunless days (1 hr. or less)	9	7	5	1	3	3
No. of absolutely sunless days	7	5	2	1	1	3
Mean max. daily temp. ° F.	54·5	59·2	65·9	75·5	69·3	66·9
Mean min. night temp. ° F.	38·8	41·7	47·3	52·6	51·9	45·0

The weather conditions during the season of 1928 were not so favourable to growth on pasture as those of the previous season. The total rainfall from April to September (inclusive) amounted to 10·1 in., compared with 13·1 in. over the corresponding period of 1927. Indeed, the present season was rendered noteworthy by the prevalence of droughty conditions at three distinct stages. April was an unusually dry month. Droughty weather was again encountered during July, since although the total rainfall for this month was satisfactory, this fell mainly in the last week, the period from July 6 to July 25 (inclusive) being, with the exception of 0·01 in. of rain on July 8, absolutely rainless. This dry period was further characterised by excessive heat and sunshine, the conditions as a whole being entirely unfavourable to the growth of herbage on light-land pasture at this time of the year. Droughty and sunny conditions were again prevalent in the final stages of the experiment, less than 0·6 in. of rain falling during September. Indeed, rainfall was registered on only four days during this month, a state of affairs in respect of weather which has not been met with before in this series of pasture investigations.

Seasonal variations in the chemical composition of the 3-weekly pasture cuts.

In Table II are summarised the data which were obtained in the analysis of the different pasture grass composite samples. The figures constitute a continuous record of the composition of the 3-weekly-cut grass from the beginning of cutting in early April to the completion of the experiment at the commencement of October. The analytical data have been corrected for small amounts of soil inevitably included in the grass samples during cutting operations, the result of such corrections being to base the figures on a common silica content of 1·72 per cent. The reasons for the adoption of the latter figure have been explained in a previous communication (1). It is not claimed that this value represents the actual percentage of silica in soil-free herbage. Indeed, during the carrying out of the present year's work, a still lower value, namely 1·38 per cent., was recorded for the silica content of the grass cut between May 9 and May 15 (inclusive). It should also be pointed out that the extent of contamination of the pasture samples by worm casts was

Table II. *Composition of soil-free samples of pasture grass from light-land pasture under system of 3-weekly cuts (on basis of dry matter).*

Digestion period		1		2		3	
Date of cutting (1928)	Apr. 12–24	Apr. 25–May 8	May 9–15	May 16–29	May 30–June 9	June 10–23	June 24–July 7
	%	%	%	%	%	%	%
Crude protein	24·89	22·69	20·64	19·53	19·73	19·29	21·34
Ether extract	6·60	6·51	5·85	6·11	5·65	6·26	5·48
N-free extractives	45·36	46·47	48·94	48·40	46·93	47·03	45·95
Crude fibre	14·28	15·26	15·72	17·00	18·50	18·82	18·38
Ash	8·87	9·07	8·85	8·96	9·19	8·60	8·85
True protein	21·55	19·89	17·74	16·84	17·56	17·18	18·85
"Amides"	3·34	2·80	2·90	2·69	2·17	2·11	2·49
Lime (CaO)	1·43	1·42	1·41	1·39	1·41	1·36	1·52
Phosphate (P_2O_5)	1·04	1·01	1·03	1·00	1·04	1·04	1·09
Moisture as cut	76·50	78·90	78·10	80·80	78·30	78·00	77·70

Digestion period	4		5		6	
Date of cutting (1928)	July 8–17	July 18–Aug. 7	Aug. 8–21	Aug. 22–Sept. 4	Sept. 5–18	Sept. 19–Oct. 3
	%	%	%	%	%	%
Crude protein	20·67	19·78	22·83	22·33	21·08	20·08
Ether extract	6·05	5·74	6·05	6·23	6·00	5·93
N-free extractives	46·12	48·31	43·38	44·69	47·46	47·82
Crude fibre	17·92	17·55	18·45	17·37	16·30	17·48
Ash	9·24	8·62	9·29	9·38	9·16	8·69
True protein	18·30	17·52	20·04	20·31	18·77	18·02
"Amides"	2·37	2·26	2·79	2·02	2·31	2·06
Lime (CaO)	1·63	1·61	1·58	1·54	1·48	1·56
Phosphate (P_2O_5)	1·06	1·08	1·20	1·20	1·20	1·17
Moisture as cut	74·20	72·60	78·10	78·30	71·10	68·90

exceedingly small throughout the whole of this season's experiment, the necessary corrections being, therefore, correspondingly insignificant.

COMMENTS ON TABLE II.

The significance of the data recorded in Table II will best be appreciated by comparing them with the results which were obtained during 1925 and 1927, under systems of weekly and fortnightly cutting respectively. This comparison is set out in detail in Table III, together with the data in respect of the composition of the 1928 weekly pasture cuts.

Table III. *Showing comparison between composition of pasture grass from light-land pasture plot under weekly, fortnightly and 3-weekly systems of cutting (dry matter basis).*

Season	1925 (3)			1927 (3)		
System of cutting	Weekly			Fortnightly		
	Extreme values		Mean value	Extreme values		Mean value
	%	%	%	%	%	%
Crude protein	21·20	27·92	24·74	21·31	27·93	23·48
Ether extract	4·72	6·45	5·29	5·76	7·55	6·53
N-free extractives	42·04	52·11	44·79	41·67	47·61	44·53
Crude fibre	12·33	17·68	15·39	12·91	17·23	15·94
SiO_2-free ash	7·26	8·72	7·77	6·88	8·82	7·80
True protein	18·13	23·85	20·95	19·18	25·26	20·84
"Amides"	2·20	5·62	3·79	2·12	3·40	2·64
Lime (CaO)	1·23	1·81	1·53	1·29	1·68	1·50
Phosphate (P_2O_5)	0·87	1·14	1·03	1·01	1·29	1·15
Moisture as cut	69·00	80·50	76·00	72·50	79·80	77·20
Season	1928			1928		
System of cutting	Weekly			3-weekly		
	Extreme values		Mean value	Extreme values		Mean value
	%	%	%	%	%	%
Crude protein	21·39	27·21	23·72	19·29	24·89	21·14
Ether extract	5·54	7·00	6·07	5·48	6·60	6·04
N-free extractives	42·44	47·38	44·97	43·38	48·94	46·68
Crude fibre	15·60	17·25	16·54	14·28	18·82	17·16
SiO_2-free ash	6·57	7·71	6·98	6·88	7·66	7·26
True protein	19·00	24·02	20·80	16·84	21·55	18·66
"Amides"	1·53	3·55	2·92	2·02	3·34	2·48
Lime (CaO)	1·29	1·67	1·45	1·36	1·63	1·49
Phosphate (P_2O_5)	0·97	1·18	1·05	1·00	1·20	1·09
Moisture as cut	67·30	78·30	74·40	68·90	80·80	76·30

The results of the trials carried out during 1925 and 1927 led to the conclusion that there was little to distinguish the composition of the herbage grown under a system of weekly cutting from that grown under a system of fortnightly cuts. The falling off in the percentage of protein

in the fortnightly-cut grass was but slight and was rather to be attributed to the less vigorous growth of wild white clover in the plot during 1927 than to any significant change in the composition of the grasses. The crude protein content of the 1928 herbage, mown at 3-weekly intervals, displayed a further slight fall, the mean value for the season being 21·14 per cent. as compared with 23·48 and 24·74 per cent. for 1927 (fortnightly cutting) and 1925 (weekly cutting) respectively. It is necessary to decide, however, how far this further decline was due to the lengthening of the interval between successive cuttings to three weeks, and how far to the influence of the peculiar weather conditions of 1928.

The influence of season is discerned by comparing the results for 1925 (weekly cutting) with those obtained in the present experiment for the pasture samples from the weekly sub-plot. The mean crude protein content of the 1928 weekly-cut herbage was slightly lower than that of the weekly-cut herbage of 1925, the respective values being, on the basis of dry matter, 23·72 and 24·74 per cent. This difference is to be ascribed to the influence of the dry weather which prevailed at three stages of the present season of experiment, since it has been demonstrated that one of the most noticeable effects of drought on pasture grass is the lowering of crude protein to which it gives rise. This effect was most outstanding during the abnormally dry September, at which stage the weekly-cut herbage suffered a considerable set-back, the percentage of crude protein falling suddenly from 27·21 to 21·40 per cent. The percentage of "amides" sank at the same time to the low level of 1·53 per cent., an indication of the almost complete cessation of growth on the weekly sub-plot. On the other hand, during the corresponding month of 1925, the crude protein content of the weekly-mown grass was as high as 26·55 per cent.

Though the influence of weather on crude protein content is not so marked in the case of grass cut at 3-weekly intervals as with weekly-cut grass, it is justifiable to conclude that the falling off of the percentage of crude protein in the 1928 3-weekly-mown herbage, as compared with the weekly and fortnightly-mown herbage of 1925 and 1927 respectively, was in some measure due to the protein-depressing influence of the droughty periods of the 1928 season. That the influence of the more lenient system of cutting was also a contributory factor, however, is seen by comparing the data for the percentages of crude protein in the herbage from the weekly and 3-weekly plots during the present season, the mean values being respectively 23·72 and 21·14 per cent. A corre-

sponding difference in respect of the percentages of true protein will also be noted.

Further definite effects which can be traced, either wholly or in part, to the adoption of a more lenient system of cutting are the slight raising of the percentages of crude fibre and of N-free extractives. On the other hand, no corresponding effect was noted in respect of ether extract, SiO_2-free ash, lime and phosphate, the percentages of these constituents being very similar in the weekly and 3-weekly-mown herbage of 1928. No useful purpose is gained by comparing the two sets of data for the moisture content of the grass samples as cut, since these values were largely influenced by the weather conditions at the time of cutting, and whereas the weekly sub-plot was cut every seven days, the cutting of the 3-weekly sub-plots was proceeding daily throughout the season.

It will not be necessary to deal at great length with the seasonal variations of the chemical composition of the pasture cuts during the present season of experiment, since in the main the results in this respect were in harmony with the conclusions already drawn from the earlier investigations of this series. The percentage of crude protein in the dry matter of the grass from the 3-weekly sub-plots displayed its maximum value during the first cutting period in April. Indeed, this early spring figure, namely, 24·89 per cent., compared quite favourably with the values which have been obtained for grass cut at weekly and at fortnightly intervals. During May, the crude protein content underwent a gradual and progressive diminution, the minimum value of 19·29 per cent. being recorded in June. Thereafter, the normal improvement in protein content was manifested, although this change received a setback in consequence of the dry weather encountered in July. The more favourable weather conditions of late July and August were reflected in an improved percentage of crude protein in the grass. This improvement, however, was again only temporary, the droughty weather which prevailed during September being responsible for another falling off in the final stages of the trial.

The depressing influence of drought on the crude protein content of pasture herbage appears to be more pronounced with grass cut at weekly intervals than with grass grown under a system of 3-weekly cuts. The September drought caused the percentage of crude protein in the herbage from the weekly sub-plot to fall abruptly from 27·21 to 21·40 per cent., the corresponding fall in the case of the 3-weekly-cut herbage being merely from 22·33 to 20·08 per cent. In further relation to this question of the influence of drought, it will be shown in later sections of this

paper that the depression of both digestibility and yield of pasture herbage, which is the accompaniment of inadequate rainfall, also becomes more pronounced as the system of cutting is intensified.

The seasonal variations of the crude fibre content of the grass were also such as would be anticipated from the results of previous investigations. The April grass contained the lowest percentage of fibre. As the season advanced, the fibre content increased progressively, the maximum percentage being recorded in June, during the period when the protein content had attained its minimum value. During the second half of the season, the tendency was towards progressively lower values for the percentage of fibre in the grass, although it is interesting to note that the September drought led to a distinct rise in this respect in the final cutting period.

In respect of the seasonal variation of the mineral composition of the grass samples from the 3-weekly sub-plots, it will be seen from the data in Table II that the maximum percentage of lime was met with in July, whereas phosphoric acid, which displayed only minor variations in amount right up to August, underwent a sudden rise in this month from 1·08 to 1·20 per cent. This new level was maintained throughout the remainder of the trial, the drought of September apparently being without significant effect on the phosphate content of the 3-weekly-cut grass. The behaviour in this regard of the grass from the weekly sub-plot was not very dissimilar, a sudden rise from 1·00 to 1·18 per cent. being noted during August. The effect of the September drought, however, was much more obvious, the phosphate content of the weekly-cut grass falling at this stage from 1·18 to 0·97 per cent.

Before dismissing this phase of the subject, it should be pointed out that the results of the present and of earlier investigations have been obtained from pasture which has received no nitrogenous fertilisers. Even the fertilising effect of the excreta of animals has been lacking during the seasons of experiment. Whether the periodic dressing of the pasture plot during the season with quickly available nitrogenous fertilisers would influence appreciably the amount, and the seasonal variations, of the protein constituent of the grass is a question which the writers hope to investigate at some future date.

Seasonal variations in the nutritive value of the pasture cuts.

The seasonal variations in the digestibility of the herbage obtained from the pasture plot under the system of 3-weekly cuts were followed by carrying out a succession of digestion trials with sheep. Six separate

digestibility determinations were made in all during the course of the investigation. As the technique of such trials has been described in detail in previous investigations, it will not be necessary to enter into such particulars in the present account. Two pure-bred Suffolk wether sheep, aged about 13 months at the commencement of the investigation, were employed in the trials, and each animal received a daily ration of 4000 gm. of the pasture herbage. During the rest intervals between experimental periods, the sheep were still maintained on the weighed diet of pasture grass.

The data relative to the digestion trials are recorded in detail in Appendix I. From these figures, it is not difficult to calculate the percentage composition of the composite samples of faeces and grass appertaining to the separate digestion trials, and for that reason, it will not be necessary to tabulate such data at this point. The mean composition of the soil-free pasture samples corresponding with the several digestion periods has already been given in Table II. The mean results for the digestion coefficients of the pasture grass are summarised in Table IV.

Table IV. *Summary of digestion coefficients (mean for two sheep).*

Period	1	2	3	4	5	6
Date (1928)	Apr. 25–May 8	May 16–29	June 10–23	July 8–17	Aug. 8–21	Sept. 5–18
	%	%	%	%	%	%
Organic matter	82·1	81·3	79·5	78·6	78·1	77·6
Crude protein	80·5	78·1	78·4	79·4	80·2	78·8
Ether extract	60·5	61·4	58·3	48·4	50·5	48·6
N-free extractives	86·2	85·8	82·7	82·2	81·0	80·8
Crude fibre	81·1	79·4	79·5	78·5	77·5	77·4

COMMENTS ON TABLE IV.

The question of primary interest in connection with the data recorded in Table IV is to inquire how these results compare with the corresponding results obtained on the same pasture in 1925 and 1927 under systems of weekly and fortnightly cutting respectively. A comparison of the digestion coefficients of the organic matter in the pasture samples under the three systems of cutting is set out in Table V.

Table V. *Digestion coefficients of organic matter of pasture cuts. Comparison of results obtained in* 1925, 1927 *and* 1928 *on light-land pasture.*

Month	Apr.	May	June	July	Aug.	Sept.
	%	%	%	%	%	%
1925 weekly cuts	83·4	83·6	80·7–77·4	74·0	74·4–75·8	79·7
1927 fortnightly cuts	82·3	81·4	78·6	78·0	78·3	79·3
1928 3-weekly cuts	82·1	81·3	79·5	78·6	78·1	77·6

The data in Table V demonstrate very clearly that, so far as digestibility is concerned, there is little to distinguish pasture grass grown under systems of weekly, fortnightly and 3-weekly cuts. Any slight differences which may be detected are capable of explanation on the basis of the different sets of meteorological conditions which characterised the three seasons of experiment.

The grass obtained during the April and May of the present experiment was just as highly digestible as that secured over the corresponding periods of 1925 and 1927 under the severer systems of cutting, and this despite the fact that the April of 1928 was a month of unusually low rainfall.

During the mid-season of 1925, under the most severe system of cutting, the digestion coefficient of the organic matter of the herbage sank to the relatively low level of 74 per cent. in consequence of the droughty conditions which prevailed at that period of the season. Although similarly dry weather conditions were also experienced at different periods of the 1927 and 1928 seasons, the depressing effect on the digestibility of the grass was not nearly so pronounced. Thus, during 1927, scarcity of rain in May did not occasion any marked falling off in the digestibility of the fortnightly-cut herbage. During the present season of experiment, droughty conditions were prevalent during mid-July. No rainfall was registered from July 6 to July 25 (inclusive), the dry matter in the pasturage as consumed by the sheep rising to 28·5 per cent. Indeed, the heat during this dry period was so excessive, that it was deemed advisable to remove the animals from the metabolism crates after ten, instead of fourteen, days of experiment and to keep them on a changed diet for several days in a shady, out-door pen. Despite the hot and droughty conditions, however, the digestibility of the 3-weekly-cut herbage remained at the high level of 78·6 per cent. Again, during the abnormally dry September of 1928, the digestion coefficient of the organic matter of the grass did not fall below 77·6 per cent.

From the foregoing observations it may be inferred that the depressing effect of drought on the digestibility of pasture herbage becomes less prominent as the system of cutting becomes more lenient. This conclusion provides an interesting corollary to the fact which has been demonstrated in previous investigations, and which is further emphasised by the results of the present trial (see a later section), that droughty conditions give rise to a more drastic depression of the productivity of a pasture under a severe system of cutting than under a lenient system. It follows, therefore, that intensification of the system of cutting renders

a pasture more liable, during periods of low rainfall, to serious set-backs not only in respect of productivity, but also in respect of the digestibility of its herbage. The significance of these conclusions in actual grazing practice will be discussed in a later section of this paper.

Table VI. *Digestion coefficients of crude protein of pasture cuts. Comparison of results obtained in* 1925, 1927 *and* 1928 *on light-land pasture.*

Month	Apr.	May	June	July	Aug.	Sept.
	%	%	%	%	%	%
1925 weekly cuts	79·3	85·4	81·1–78·4	77·1	76·6–78·9	83·4
1927 fortnightly cuts	81·7	79·3	78·8	79·2	79·5	80·7
1928 3-weekly cuts	80·5	78·1	78·4	79·4	80·2	78·8

It will be concluded from Table VI that although the grass obtained during 1928 under a system of 3-weekly cuts was somewhat less rich in crude protein than that obtained under the more severe systems of cutting adopted in 1925 and 1927, this minor change in composition was unaccompanied by any corresponding reduction of the digestibility of the protein constituent. When allowances are made for the differences in the meteorological conditions experienced in the three seasons of experiment, and for the fact that different pairs of animals were employed in the separate investigations, it is justifiable to conclude that the digestibility of the crude protein of the grass is the same, whether the grass be grown under a system of weekly, fortnightly or of 3-weekly cuts. It is interesting to observe that the stage in the growth of grass where the protein content begins to fall is reached before the point at which digestibility and nutritive value begin to run off. Indeed, it is possible, though experimental confirmation must await future investigations, that the percentage of protein in the grass may display quite a considerable falling off before the stage of growth is reached which marks the decline in digestibility and nutritive value. This, and kindred questions, cannot, however, be elucidated by mere chemical analysis of pasture grass in its various stages of development. The necessity of supplementing such investigations by means of digestion trials is manifest.

Attention should further be directed to the fact that the seasonal variations of protein digestibility were not very conspicuous, despite the meagreness of the rainfall during April, July and September.

The data in Table VII call for little comment, since the general similarity between the values for the digestion coefficients of the ether extract of the weekly, fortnightly and 3-weekly-mown herbage is at once

apparent. The trend of seasonal variation was slightly different in the three separate investigations, but this difference can readily be correlated with the seasonal variations of the rainfall for the respective seasons. The reduction in the digestibility of the ether extract of pasture grass which is brought about by droughty conditions is again exemplified in the lower values of the digestion coefficients for July and September of the present investigation. It will also be noted that the widest range of variation was experienced in the season of most severe cutting.

Table VII. *Digestion coefficients of ether extract of pasture cuts. Comparison of results obtained in* 1925, 1927 *and* 1928 *on light-land pasture.*

Month	Apr. %	May %	June %	July %	Aug. %	Sept. %
1925 weekly cuts	60·5	60·0	53·5–51·2	39·7	47·9–55·1	54·9
1927 fortnightly cuts	66·0	59·2	52·7	54·6	53·7	51·9
1928 3-weekly cuts	60·5	61·4	58·3	48·4	50·5	48·6

Table VIII. *Digestion coefficients of* N-*free extractives of pasture cuts. Comparison of results obtained in* 1925, 1927 *and* 1928 *on light-land pasture.*

Month	Apr. %	May %	June %	July %	Aug. %	Sept. %
1925 weekly cuts	88·1	87·4	81·6–78·5	75·0	76·0–77·4	79·9
1927 fortnightly cuts	85·6	85·3	80·8	80·4	80·0	82·0
1928 3-weekly cuts	86·2	85·8	82·7	82·2	81·0	80·8

The figures in Table VIII emphasise still further the similarity, in respect of digestibility, of pasture grass cut at weekly, fortnightly and 3-weekly intervals, the digestion coefficients of the N-free extractives of the grass, apart from differences connected with seasonal variation, being very similar in all three investigations. It will specially be noted that conditions of drought were responsible for a very much bigger reduction of the digestibility of the N-free extractives under the weekly system of pasture cuts in 1925 than under the system of 3-weekly cuts adopted during the present trial.

Table IX. *Digestion coefficients of crude fibre of pasture cuts. Comparison of results obtained in* 1925, 1927 *and* 1928 *on light-land pasture.*

Month	Apr. %	May %	June %	July %	Aug. %	Sept. %
1925 weekly cuts	80·3	79·2	84·2–80·0	76·1	76·0–75·1	80·4
1927 fortnightly cuts	82·1	81·1	81·3	80·3	82·3	81·1
1928 3-weekly cuts	81·1	79·4	79·5	78·5	77·5	77·4

That pasture grass grown under a system of 3-weekly cuts is equal to weekly and fortnightly-mown grass in respect of digestibility is demonstrated conclusively by the figures recorded in Table IX. The fibre digestion coefficients of the 1928 herbage, despite a slight rise in the actual crude fibre content of the grass, compare very satisfactorily with the corresponding data obtained in the 1925 and 1927 investigations. Under a system of 3-weekly cuts, therefore, pasture grass never reaches the stage of growth at which lignification sets in, with consequent decline in the digestibility not only of the fibrous component, but also of the other organic constituents. The crude fibre of pasture herbage, after three weeks' unchecked growth, still consists mainly of digestible cellulose, unmixed with any appreciable proportion of the more carbonaceous and entirely indigestible lignocellulose.

The foregoing considerations render justifiable the conclusion that the differences in digestibility between pasturage cut at weekly, fortnightly and 3-weekly intervals are quite inconspicuous. At the end of a 3-weeks' period of growth, pasture grass still retains the non-lignified, highly digestible character which it possessed at the end of a week's or of a fortnight's growth. Moreover, the characteristic of high digestibility, by cutting at 3-weekly intervals, is maintained throughout the entire season. It may be inferred, therefore, that similar results would follow from a system of rotational grazing, where the pasture enclosures, after being thoroughly eaten down by stock, are permitted a 3-weeks' interval of unchecked growth before being grazed again. It will be the object of future investigations to discover the extent to which the interval between successive grazings may be further lengthened without causing the herbage to lose its highly digestible character.

It will be of interest at this point to note some of the observations which were made of the botanical characteristics of the herbage during the present season of experiment and to point out, in particular, the stages of growth to which the main forms of herbage attained at the different stages of the season[1].

One of the outstanding botanical features of this series of investigations is the manner in which *Agrostis alba v. stol.* (creeping bent) has, season by season, increased in importance under the systems of periodic cutting to which the plot has been submitted during the 1925, 1927 and 1928 seasons. At the commencement of the trials in 1925, creeping bent

[1] The writers again have pleasure in recording their gratitude to their colleague, Mr S. F. Armstrong, M.A., for valuable assistance in connection with the botanical phases of the investigation.

was by no means conspicuous among the grasses of the plot. It became early apparent, however, that frequent cutting would favour the spreading and development of this creeping species, just as it also favoured the growth of wild white clover. By the end of the 1925 trial, creeping bent had already established itself as one of the important species in the plot, and at the present time it may be said to have gained the position of dominance. During the spring of the present season its importance was second only to that of perennial rye grass. At the beginning of July, creeping bent took precedence of rye grass and towards the end of August it was estimated that this species formed quite half of the herbage which was being cut and fed to the sheep.

These facts are not without significance from the nutritional standpoint. It has previously been demonstrated (2) that under conditions of close grazing, creeping bent approximates to grasses like perennial rye grass in respect of composition, digestibility and nutritive value. The results of the present investigation demonstrate further that under a system of rotational grazing, where the herbage is grazed closely at 3-weekly intervals, pasture herbage containing a high proportion of creeping bent is not inferior, from the standpoint of digestibility, to any other type of herbage. The best results are clearly to be obtained from pastures when the conditions are such as to permit of the herbage being grazed down *closely* at suitable intervals, as in rotational grazing practice.

During the first half of May in the present trial, the main grass in the plot, namely, perennial rye grass, was just entering the stage of spike emergence, with leaves up to 4 in. in height and flower stems up to 6 in. Creeping bent at this date consisted of young foliage only, the shoots varying from 1 to 3 in. in height. Rough-stalked meadow grass was also prominent in the form of young tender leaves of height from 1 to 3 in. The young leafage of wild white clover, height from 1 to 2 in., was plentifully distributed throughout the pasture plot. Of the weeds, which, with the exception of dandelion heads, were included in the pasture samples for analysis and for feeding, bulbous buttercup (6 in. high), daisies (3 in. high) and dandelions (3 to 8 in. high) were in the full flowering stages. Yarrow and burnet (2 to 3 in. high) were still in the young leafy stages, whereas sweet vernal grass was entering the flowering phase, the height of the flower stems varying from 3 to 6 in.

By the end of May, it was noted that the flowering stems of perennial rye grass were much reduced in number, both rye grass and creeping bent being present mainly in the form of foliage. Rough-stalked meadow

grass had reached the pre-flowering stage (stems 2 to 6 in. long) and its flowering culms, though small, were abundant. Buttercups and daisies were still flowering, though only the leaves of dandelion were now in evidence. Meadow foxtail had already advanced past the stage of flowering. Sweet vernal grass was flowering, while burnet and golden oat grass were in the pre-flowering stage.

A further botanical inspection on June 22 showed that perennial rye grass was still represented mainly by foliage, whereas creeping bent had arrived at the pre-flowering stage. Wild white clover was increasing in amount and cocksfoot was more conspicuous than formerly. Neither of these species had so far flowered under the conditions of the experiment. Golden oat grass was now flowering abundantly, as also was rough-stalked meadow grass, though to a smaller extent. It was noted that the "sole" of the pasture was now thick and dense with mixed leafy herbage, which had a fresh green appearance and was in ideal condition for grazing. The proportion of plants flowering appeared to be low in comparison with what had been observed during the previous month. At this stage, the "top" grasses were from 3 to 5 in. in height.

Wild white clover was found to be flowering on the occasion of the next detailed survey on July 6. Creeping bent had further increased in amount and consisted mainly of foliage with some flowering stems, while perennial rye grass, though not quite so abundant as in the earlier stages of the season, still continued to be characterised by its leafiness. Red fescue foliage was now quite conspicuous and the amount of cocksfoot had also displayed further increase. Yorkshire fog, represented mainly by leafage with but few flowering stems, was now to be noted in the plot. At this stage, the herbage appeared to be slowly losing its early vigour of growth. The "top" grasses were growing to a height of 3 to 5 in.

The date of the next botanical inspection was July 26, which marked the end of three weeks of hot, rainless days. On this date, the whole pasture presented a rather "scorched" appearance, and the herbage was short and scanty. Of the grasses, only cocksfoot, red fescue and Yorkshire fog were looking fresh. The remaining species, particularly creeping bent and rough-stalked meadow grass, were considerably "scorched." Nothing was flowering except a few plantains and hawkweeds.

Under the influence of the rainfall of late July and August, the herbage had regained its fresh green and growing appearance at the time of the next botanical survey on August 24. Creeping bent was now the predominant species and constituted something like 50 per cent. of the pasturage which was being cut and fed to the sheep. Weeds had

increased somewhat in amount and wild white clover was displaying its maximum growth for the season, forming about 10 per cent. of the mown herbage. The "top" grasses were 2 to 3½ in. in height. None of the grass species was flowering.

The prolonged drought of September led to herbage becoming short and scanty again. The plot took on a "browned" appearance, creeping bent in particular suffering from scarcity of rainfall. It was noted, however, that only a small proportion of the "browned" herbage found its way into the pasture samples secured by means of the lawn mower for purposes of analysis and feeding to the experimental animals. At the commencement of October, the productivity of the pasture plot had fallen almost to zero level in consequence of the late seasonal drought.

The foregoing botanical observations have been recounted in some detail in order to give the reader an opportunity of considering the results of the digestion trials in relation to the stages of growth to which the herbage was able to attain under the conditions of the experiment. One fact emerges clearly, namely, that under the system of cutting at 3-weekly intervals, the herbage was unable to advance far in maturity and retained, for the most part, the appearance of leafiness which is characteristic of young grass in its vegetative and pre-flowering stages of growth. The fact that the pasturage maintained a high level of digestibility throughout the entire season is quite in harmony with the botanical findings.

Table X. *Amounts of digestible nutrients in dry matter of pasture cuts. Summary of starch equivalents and nutritive ratios.*

Period	1	2	3	4	5	6
Date (1928)	Apr. 25–May 8	May 16–29	June 10–23	July 8–17	Aug. 8–21	Sept. 5–18
	%	%	%	%	%	%
Digestible crude protein	18·27	15·25	15·12	16·41	18·31	16·61
Digestible ether extract	3·94	3·75	3·65	2·93	3·06	2·92
Digestible N-free extractives	40·06	41·53	38·89	37·91	35·14	38·35
Digestible crude fibre	12·38	13·50	14·96	14·07	14·30	12·62
Digestible organic matter	74·65	74·03	72·62	71·32	70·81	70·50
P.S.E. per 100 lb. dry matter	72·72	71·60	69·59	67·82	67·15	67·44
Nutritive ratio	3·37	4·17	4·12	3·58	3·08	3·47

Comments on Tables X and XI.

An inspection of the data recorded in Table XI reveals the fact that the widening of the interval between successive cuts to three weeks has led to a slight fall in the digestible protein content of the pasture herbage, with a corresponding slight increase in the content of digestible

carbohydrate. The fall in the percentage of digestible protein, however, is unaccompanied by any corresponding diminution in the percentage of total digestible organic matter in the grass, or of the value of the starch equivalent per 100 lb. of dry matter. The excellent agreement in respect of digestible organic matter and starch equivalent between the fortnightly-cut herbage of 1927 and the 3-weekly-cut herbage of 1928 will be noted. Indeed, such differences in these respects as are discernible between the data for 1925, 1927 and 1928 are to be attributed to differences of seasonal variation, consequent on the different meteorological conditions experienced in the three seasons, rather than to a direct effect connected with the different systems of cutting. It may be concluded that grass obtained under a 3-weekly cutting system, while slightly less rich in digestible protein, is nevertheless equal in respect of total digestible organic matter and starch equivalent to grass grown under weekly and fortnightly systems of cutting.

Table XI. *Showing comparison between digestible composition of herbage from light-land pasture plot under weekly, fortnightly and 3-weekly systems of cutting (on basis of dry matter).*

Season	1925 (3)			1927 (3)			1928		
System of cutting	Weekly			Fortnightly			3-weekly		
	Extreme values		Mean value	Extreme values		Mean value	Extreme values		Mean value
	%	%	%	%	%	%	%	%	%
Digestible crude protein	16·25	23·45	19·97	17·09	20·82	18·75	15·12	18·31	16·66
Digestible ether extract	1·96	3·55	2·87	3·16	4·98	3·81	2·93	3·94	3·38
Digestible N-free extractives	33·30	45·91	36·10	33·50	40·38	36·50	35·14	41·53	38·65
Digestible crude fibre	9·48	14·14	12·08	11·54	14·10	13·10	12·38	14·96	13·64
Digestible organic matter	66·89	75·59	71·02	70·20	74·54	72·16	70·50	74·65	72·33
P.S.E. per 100 lb. dry matter	62·08	73·70	67·74	67·79	73·75	69·87	67·15	72·72	69·39
Nutritive ratio	2·18	3·72	2·79	2·89	3·59	3·13	3·08	4·17	3·63

In previous communications (1, 2, 3), it has been shown that grass cut at weekly and fortnightly intervals is too rich in digestible protein to provide, *when it constitutes the sole ingredient of the diet*, a correctly balanced diet for any class of farm animal. The desirability of employing carbohydrate concentrates, instead of oil cakes, for supplementary feeding on closely-grazed pastures has accordingly been emphasised. It will now be noted, from the data in Table XI, that the nutritive ratio of the pasture herbage is gradually becoming wider as the system of cutting becomes less severe. Nevertheless, the nutritive ratio of the grass obtained during the early part of the 1928 season was still significantly narrower than that of milk. This was also true of the pasture

herbage during July, August and September. Manifestly, therefore, in respect of the grass obtained during these periods of the season, there exists no reason for modifying the conclusion arrived at in the 1925 and 1927 investigations respecting supplementary feeding on pastures.

During May and June of the present season of experiment, the nutritive ratio of the pasture grass widened to a value almost equal to the nutritive ratio of milk, namely, 4·2. It may be concluded, therefore, from the standpoint of digestible protein content, that the grass over this period would be adapted to meeting, in itself, the requirements of young animals. In the case of dairy animals, it is recognised that cows yielding from 3 to 5 gallons of milk per day require, according to the scientific standards, a ration in which 1 part of digestible protein is associated with 5 to $5\frac{1}{2}$ parts of starch equivalent. Since the May–June herbage contained 1 part of digestible protein in about 4·6 parts of starch equivalent, it is clear that the grass in this part of the season was approaching the balance suitable for such dairy animals. For fattening stock, however, it would still be necessary, in order to furnish a diet balanced according to scientific standards, to supplement the pasturage at this date with some carbohydrate-rich food. A 13 cwt. fattening steer, for instance, subsisting on a ration of such grass containing 28 lb. of dry matter, would consume daily about 20 lb. of starch equivalent, including 4·3 lb. of digestible protein. Its actual requirements, according to the scientific standards of feeding, are 15·9 lb. of starch equivalent including only 1·8 lb. of digestible protein.

The results obtained in the present investigation respecting the nutritive ratio of pasture grass cut at 3-weekly intervals encourage the hope that grass grown under a system of monthly cuts, while retaining the high digestibility and nutritive value associated with concentrated foods, will also contain digestible protein and digestible non-protein constituents in such proportions as to render it much more suited to form the sole diet of farm animals. This question will be submitted to investigation in the coming season, and the results should possess a particular interest, in that a period of four weeks has been commonly adopted in this country as the interval which is allowed to elapse, in rotational grazing practice, between successive grazings of enclosures.

In considering the significance of the results, it is again advisable to point out that this series of investigations is being conducted on a pasture which, apart from a dressing of basic slag in 1925, has not been fertilised for many years. It is conceivable that the application of the new system of nitrogenous fertilising on pastures, while leading to a

more abundant growth of herbage, might also reduce the range of seasonal variation of the content of digestible protein. It is the intention of the writers to expand the scope of these investigations in future seasons in the direction of ascertaining the influence of fertilisers on the yield, composition and nutritive value of pasture herbage. The present pasture will be eminently suitable for such further investigations, since its capacities under unmanured conditions will have been fully explored.

PRODUCTION OF NUTRIENT MATTER FROM PASTURE PLOTS UNDER SYSTEMS OF WEEKLY, FORTNIGHTLY AND 3-WEEKLY CUTTING.

In the third publication of this series (3), it was shown that if contiguous plots on a pasture be submitted to cutting at weekly and fortnightly intervals respectively, then the fortnightly-cut plot will be found to yield somewhat more heavily than the plot cut every week. Further, this disparity in productivity becomes most marked at those times of the season when the conditions for growth are most unfavourable, as, for instance, during a spell of droughty weather. As will be made clear later (see Table XV) the weather conditions of the season of the present experiment were, on the whole, unfavourable to the abundant growth of herbage on pastures, and it would therefore be anticipated that the yields from the sub-plots submitted to weekly, fortnightly and 3-weekly systems of cuts would display unusually striking differences. That this was actually the case is made evident by Table XII, in which a comparison is given of the total yields of herbage, in lb. dry matter per acre, which were obtained from (1) the 3-weekly-cut experimental pasture plot, (2) the sub-plots cut at weekly and fortnightly intervals respectively, and (3) the sub-plot from which a hay cut (June 12) and a single late aftermath cut (October 2) were taken. The positions of the sub-plots, in relation to the main 3-weekly pasture plot, have already been noted in an earlier section of this paper.

Table XII. *Production of dry matter from light-land pasture plot under different systems of cutting during* 1928.

System of cutting	lb. dry grass matter per acre
Weekly (Apr. 12 to Oct. 3)	1982
Fortnightly (Apr. 12 to Oct. 3)	2562
3-weekly (Apr. 12 to Oct. 3)	3216
Meadow sub-plot: Hay cut (June 12) + aftermath cut (Oct. 2)	6655

It will be noted from Table XII that under the weather conditions of the grazing season of 1928, cutting at fortnightly intervals produced

29·3 per cent. more dry matter than was obtained under a system of weekly cuts, whilst the yield obtained by cutting at 3-weekly intervals was 62·3 per cent. greater than that obtained by weekly cutting and 25·5 per cent. greater than that grown under a fortnightly cutting system. These yield differences are not to be explained on the basis of lack of uniformity in respect of productive capacity among the sub-plots. This point was tested thoroughly in the 1925 season, with the results which are recorded in Table XIII.

Table XIII. *Yield over whole season from weekly-cut sub-plots during* 1925 (1).

Sub-plot	1	2	3	4	5	6	7
Yield in lb. dry matter per $\frac{1}{4}$ acre	452	437	441	448	431	426	430

It will be noted from Table XIII that the experimental pasture is reasonably uniform in respect of productivity, the difference in yield of the best and worst sub-plots being about 6 per cent.

The differences in yield from the weekly, fortnightly and 3-weekly plots during the present season are therefore in main measure to be ascribed to the influence of the different systems of cutting on productivity, a statement which receives further support by the manner in which the yield differences were exaggerated at those stages of the season when especially unfavourable growth conditions prevailed (see Table XIV).

It has already been demonstrated that there is little or no difference, from the standpoint of starch equivalent, between pasture herbage grown under systems of weekly, fortnightly and 3-weekly cuts. The yield differences under the three systems are possessed, therefore, of great practical significance. It may be assumed that a system of weekly cuts is comparable with grazing as practised in districts like the Romney Marsh, where it is the custom of the grazier to regulate the stocking of his sheep-grazing land in such a manner as to keep the herbage uniformly grazed down throughout the duration of the grass season. If the herbage shows any tendency to grow beyond the young stage, more sheep are introduced to hold it in check. This practice may be referred to, for convenience, as "non-rotational close-grazing."

On the other hand, a system of 3-weekly cuts may be taken as conforming with the conditions of rotational grazing, where the interval between successive close-grazings is of three weeks' duration. It will be convenient, for the purposes of the present discussion, to refer to such a system as a 3-weeks' "rotational close-grazing" system.

The yield results of the present investigation bring to light an important advantage which "rotational close-grazing" possesses over a system of "non-rotational close-grazing." To make this point clear, it is merely necessary to imagine that the pasture, on which this series of investigations is being carried out, had been so stocked during 1928 that the herbage was kept closely and uniformly grazed throughout the entire grazing season. Under such conditions, the pasture would have produced herbage, for the sustenance of the animals, at the rate of about 1980 lb. of dry matter per acre over the season. If, on the other hand, the tract of grass had been divided up into smaller areas in such a way that each enclosure, after being closely grazed by stock, was permitted a 3-weeks' interval of unchecked growth before being grazed down again, the pasture would have produced herbage at the rate of about 3220 lb. of dry matter per acre over the season. This herbage, moreover, would have been equal, in respect of starch equivalent, to that obtained in smaller amount under the system of "non-rotational close-grazing," and although it would have been somewhat less rich in digestible protein, this difference, as has been clearly demonstrated in the section dealing with digestibility and nutritive value, would have constituted an advantage rather than a drawback from the standpoint of pasturing stock not receiving supplementary food.

It may be concluded, therefore, that if the management of the experimental pasture had been attempted along the lines of close-grazing under the weather conditions of 1928, a simple division of the main field into a suitable number of smaller enclosures, for rotational grazing at 3-weekly intervals, would have enabled the stock-carrying capacity of such unfertilised pasture to be increased in the ratio of 198 : 322 (*i.e.* roughly 2 : 3). It should be emphasised, however, that this specific measure of improvement applies solely to the pasture in question under the particular meteorological conditions of the 1928 season. The magnitude of the improvement which might be effected in this way would undoubtedly be smaller in a season where the weather conditions as a whole were more favourable to growth of pasturage than those of 1928.

The foregoing results reveal, therefore, an important advantage appertaining to the practice of rotational grazing, and it will be of interest to ascertain how this factor further operates in the work which is to be carried out during the coming season, when a system of cutting at monthly intervals will be adopted.

The present yield data possess further practical significance in relation to the proposals which have been put forward (1, 2, 3) for con-

serving pasture grass, by artificial drying and pressing into cakes or by ensilage, for use as a concentrate during winter. Before adopting such proposals, it would be necessary to decide the frequency with which the grass should be cut during the growing season. The results of this year's investigation should prove of help in coming to such a decision. From the standpoints of digestibility and starch equivalent, it is immaterial whether the herbage is cut at weekly, fortnightly or 3-weekly intervals. Under the 3-weekly system, however, the season's yield of herbage will be greater than will be obtained by cutting at shorter intervals, and the difference will be accentuated during seasons when the conditions are not favourable for growth of grass. In regard to protein content, the average figure, on the dry matter basis, for weekly-cut grass will be about 25 per cent., that for fortnightly-cut grass about 23·5 per cent. and that for 3-weekly-cut grass about 21 per cent. It is hoped to secure similar information concerning grass cut at monthly intervals during the coming season.

Table XIV. *Seasonal yields of herbage from light-land pasture plot during* 1928 *under systems of weekly and* 3-*weekly cuts.*

Period of season (1928)	Mean weekly rainfall (in.)	Weekly cutting lb. dry matter per acre	3-weekly cutting lb. dry matter per acre	Difference as % of weekly-cut grass
Apr. 12 to Apr. 26	0·07	102·0	190·2	86·5
Apr. 27 to July 5	0·53	1164·0	1798·4	54·5
July 6 to July 25	0·00	86·0	281·7	228·0
July 26 to Aug. 30	0·72	546·0	635·7	16·4
Aug. 31 to Oct. 3	0·12	84·0	310·0	269·0

It has already been pointed out that, over the entire 1928 season of experiment, the 3-weekly system of cutting gave rise to 62·3 per cent. more herbage (on the dry matter basis) than was obtained by cutting every week. The yield data are analysed in slightly greater detail in Table XIV with the object of bringing out the comparative productivities of the weekly and 3-weekly plots at the different periods of the season.

The figures clearly emphasise the rule which has already been enunciated, namely, that unfavourable weather conditions, such as drought, lead to a pronounced accentuation of the disparity of yield between pasture plots submitted to lenient and to severe systems of cutting respectively. During the droughty September of 1928, for example, the 3-weekly plots produced 269 per cent. more herbage than the weekly plot. Again, during the rainless days of July, the disparity in yield amounted to 228 per cent. in favour of the 3-weekly plot. Even during the

comparatively dry fortnight between April 12 and 26, when it would not be expected that scarcity of rainfall would have exerted any pronounced depressing effect on productivity, the leniently-cut plots grew 87 per cent. more herbage than the weekly plot. On the other hand, during the two periods of the season where rainfall was adequate for active growth, namely, April 27 to July 5 and July 26 to August 30, the disparity in productivity sank to 55 and to 16 per cent. respectively.

Table XV. *Summary of total yields of herbage from the light-land pasture plot in three different seasons of experiment.*

	1925	1927	1928
Apr. 12 to beginning of October	lb. dry matter per acre	lb. dry matter per acre	lb. dry matter per acre
Weekly cutting	2833	—	1982
Fortnightly cutting	—	3621	2562
3-weekly cutting	—	—	3216

The data in Table XV serve to demonstrate the primary importance of the general weather conditions of the season on the growth of pasturage. The weather conditions of 1925 and 1927 were, on the whole, favourable to the abundant growth of pasture herbage. The season of 1928, on the other hand, was a poor pasture year, owing to the prevalence of droughty conditions at three different stages (see an earlier section).

It is scarcely surprising, therefore, that by cutting every *week*, the plot produced 42·9 per cent. more herbage during 1925 than during 1928. The influence of the unfavourable weather conditions of 1928 had a more far-reaching effect than this, however, inasmuch as the yield during this season under a system of *fortnightly* cuts was about 10 per cent. *lower* than that obtained by *weekly* cutting in 1925. To similar considerations of weather is to be ascribed the fact that the plot during 1927, under a system of *fortnightly* cutting, yielded 41·3 per cent. more herbage than was obtained under the *same* system of cutting during 1928 and 12·6 per cent. *more* than was obtained by cutting at 3-*weekly* intervals during the present season.

Within the limits of the systems of cutting which have so far been investigated, it is clear that unfavourable meteorological conditions in a particular season may lead not merely to a much smaller growth of grass than would be obtained, by the same system of cutting, in a more favourable year, but may actually cause the yield under a lenient system of cutting to be smaller, instead of larger, than was obtained under a less-lenient system of cutting during the more favourable season.

The productivity of pastures is therefore controlled primarily by

meteorological factors, of which the most important is the rainfall, in particular, its distribution over the season. If the weather conditions as a whole are unfavourable during any particular season, then the yield will be influenced considerably by the degree of intensity of cutting or grazing. Other factors which have an effect on the productivity of pastures are: (1) Manurial constituents in the soil.—The fertilising of pastures should lead to a denser and more vigorous growth of herbage. Although the present series of investigations is being conducted on unfertilised pasture, it is hoped shortly to carry out experiments to ascertain the amount of improvement which can be effected in the yield of nutrient matter by the use of fertilisers. At the same time, the related question of the rate of exhaustion of the soil in respect of nitrogen, lime and phosphate under different systems of cutting will be dealt with. (2) Physical character of soil.—A pasture on a soil of high water-retaining capacity will withstand the effects of short droughts more successfully than pasturage on a light sandy soil. (3) Botanical character of herbage.—For continuous active growth over the entire season, it is of advantage that a pasture should contain a large number of different species of grasses possessing different periods of luxuriance.

During the course of these investigations, a number of curious observations have been made respecting the influence of meteorological conditions on the growth of hay and aftermath. The results indicate that in one and the same season, weather conditions may exert quite different influences on the yields of meadow and of pasture. It is proposed, however, to await the carrying out of further investigations into the yield and composition of hay and aftermath before reporting in detail on this phase of the grassland question.

Summary.

The object of this series of investigations is to secure detailed information concerning the composition, digestibility and nutritive value of pasture grass in its different stages of growth. The results which were obtained in these respects by cutting the herbage of the experimental pasture plot at weekly and at fortnightly intervals have been described in previous communications. During the season of the present experiment, the trials have been carried a stage further by the adoption of a system of cutting at 3-weekly intervals. The main findings of the 1928 investigation are recorded below:

(1) Chemical composition of 3-weekly pasture cuts: The adoption of a more lenient system of cutting at 3-weekly intervals led to a slight

lowering of the percentage of crude protein in the grass and a slight raising of the percentages of crude fibre and N-free extractives. On the other hand, no corresponding effect was noted in respect of the ether extract, SiO_2-free ash, lime and phosphate, the percentages of these constituents being very similar in the weekly and 3-weekly pasture samples obtained in 1928. The falling off of the percentage of crude protein in the 1928 3-weekly-mown herbage, as compared with the weekly and fortnightly-mown herbage of 1925 and 1927 respectively, was not wholly the consequence of the more lenient system of cutting, but was also due in part to the protein-depressing influence of the droughty periods which were experienced in the 1928 season.

The main results relating to the seasonal variations in the chemical composition of the 3-weekly pasture cuts were in harmony with those obtained in the 1925 and 1927 investigations. The depressing influence of drought on the crude protein content of grass appears to be more pronounced with grass cut at weekly intervals than with grass grown under a system of 3-weekly cuts.

(2) Digestibility of 3-weekly pasture cuts: So far as digestibility is concerned, there is little to distinguish grass grown under systems of weekly, fortnightly and 3-weekly cuts. The depressing influence of drought on the digestibility of pasture herbage becomes less pronounced as the system of cutting becomes more lenient.

Although the grass obtained by cutting every three weeks during 1928 was somewhat less rich in crude protein than that obtained under the more severe systems of cutting adopted during 1925 and 1927, this change in composition was unaccompanied by any corresponding reduction of the digestibility of the protein constituent. Obviously the stage in the growth of grass where the protein content begins to fall is reached before the point at which digestibility and nutritive value begin to run off.

Under a system of 3-weekly cuts, pasture grass never reaches the stage of growth at which lignification sets in, with consequent decline in the digestibility of not only the fibrous constituent, but also of the other organic constituents. At the end of a 3-weeks' period of growth, pasture grass still retains the non-lignified, highly digestible character which it possessed at the end of a week's or of a fortnight's growth. Since this characteristic of high digestibility is retained, by cutting at 3-weekly intervals, over the whole season, it may be inferred that similar results would follow from a system of rotational grazing, where the pasture enclosures, after being closely-grazed, are permitted a 3-weeks' interval of unchecked growth before being grazed again.

(3) Nutritive value of 3-weekly pasture cuts: Pasture grass obtained under a system of 3-weekly cuts, while slightly less rich in digestible protein, is nevertheless equal in respect of total digestible organic matter and of starch equivalent to grass grown under systems of weekly and of fortnightly cutting. The dry matter of such herbage is still to be looked on as a protein concentrate of high digestibility and nutritive value.

Although the nutritive ratio of the pasture herbage is gradually becoming wider as the system of cutting becomes less severe, the values for the grass obtained by cutting every three weeks were still significantly narrower than that of milk during the early part of the 1928 season and also during July, August and September.

For the herbage obtained during these periods of the season, therefore, the earlier conclusions still hold good respecting the desirability of employing carbohydrate-rich foods, instead of oil cakes, for the supplementary feeding of pasturing animals. During May and June, however, the nutritive ratio of the pasturage widened to a value almost equal to the nutritive ratio of milk. At this stage, the grass was adapted to the requirements of young stock and was approaching a suitable balance for dairy cows yielding from 3 to 5 gallons of milk per day. For fattening stock, however, a supplement of carbohydrate-rich food would still be necessary in order to furnish such animals with a diet balanced according to scientific standards. The tendency of the results in this connection lends additional interest to the next stage of these trials, namely, the repetition of the work under a system of monthly cuts.

(4) Botanical findings: Under the system of cutting at 3-weekly intervals, the herbage was unable to advance far in maturity and retained, for the most part, the appearance of leafiness which is characteristic of young grass in its vegetative and pre-flowering stages of growth. The fact that the pasturage maintained a high level of digestibility throughout the entire season was quite in harmony with the botanical findings.

The results demonstrate further that under a system of rotational grazing, where the enclosures are grazed *closely* at 3-weekly intervals, pasture herbage containing a high proportion of creeping bent is not inferior, from the standpoint of digestibility, to any other type of herbage. The best results are clearly to be obtained from pastures when the conditions are such as to permit of the herbage being grazed down *closely* at suitable intervals, as in rotational grazing practice.

(5) Yield results: The yields of nutrient matter obtained from the pasture sub-plots under systems of weekly, fortnightly and 3-weekly

cuts have been measured during the present season of experiment. Cutting at fortnightly intervals produced 29·3 per cent. more dry matter over the whole season than was obtained under a system of weekly cuts, whilst the yield obtained by cutting at 3-weekly intervals was 62·3 per cent. greater than that obtained by weekly cutting and 25·5 per cent. greater than that grown under a fortnightly cutting system. The disparities in productivity were most outstanding at those periods of the season when unfavourable meteorological conditions for growth were prevalent. The significance of these findings in relation to the question of pasture grass conservation has been gone into.

It has been demonstrated that if the management of the experimental pasture had been attempted along the lines of close-grazing *under the weather conditions of* 1928, a simple division of the main field into a suitable number of smaller enclosures, for rotational grazing at 3-weekly intervals (*i.e.* a 3-weeks' "rotational close-grazing" system), would have enabled the stock-carrying capacity of such unfertilised pasture to be increased in the ratio of 2 : 3.

Within the limits of the systems of cutting which have so far been investigated, it has been shown that unfavourable meteorological conditions in a particular season may lead not merely to a much smaller growth of grass than would be obtained, by the same system of cutting, in a more favourable year, but may actually cause the yield under a lenient system of cutting to be smaller, instead of larger, than was obtained under a less lenient system of cutting during the more favourable season.

It is emphasised that the results which have so far been obtained in this series of investigations refer to pasturage under unfertilised conditions. It is hoped shortly to carry out experiments to ascertain the amount of improvement which can be effected in the yield of nutrient matter from the experimental pasture by the use of fertilisers.

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Appendix I. *Digestion tables**.

Daily ration: 4000 gm. pasture grass.

	Sheep VII							Sheep VIII						
	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
***Period* 1†**														
Consumed	—	—	—	—	—	—	—	1004·00	908·82	226·80	65·06	464·45	152·51	95·18
Voided	—	—	—	—	—	—	—	222·30	163·17	44·30	25·70	64·29	28·88	59·13
Digested	—	—	—	—	—	—	—	781·70	745·65	182·50	39·36	400·16	123·63	36·05
Digestion coefficients (%)	—	—	—	—	—	—	—	77·86	82·05	80·47	60·50	86·16	81·06	37·88
***Period* 2**														
Consumed	891·20	807·61	173·25	54·19	429·38	150·79	83·59	891·20	807·61	173·25	54·19	429·38	150·79	83·59
Voided	198·30	148·35	37·54	19·71	59·95	31·15	49·95	203·30	153·10	38·20	22·10	61·78	31·02	50·20
Digested	692·90	659·26	135·71	34·48	369·43	119·64	33·64	687·90	654·51	135·05	32·09	367·60	119·77	33·39
Digestion coefficients (%)	77·75	81·63	78·33	63·63	86·04	79·34	40·24	77·19	81·04	77·95	59·22	85·61	79·43	39·94
***Period* 3**														
Consumed	958·00	855·59	180·58	58·63	440·20	176·18	102·41	958·00	855·59	180·58	58·63	440·20	176·18	102·41
Voided	238·80	175·52	38·46	23·71	77·20	36·15	63·28	238·90	175·85	39·51	25·23	75·04	36·07	63·05
Digested	719·20	680·07	142·12	34·92	363·00	140·03	39·13	719·10	679·74	141·07	33·40	365·16	140·11	39·36
Digestion coefficients (%)	75·07	79·48	78·70	59·56	82·46	79·48	38·21	75·06	79·45	78·12	56·97	82·95	79·53	38·43
***Period* 4**														
Consumed	1140·00	1018·02	231·88	67·83	517·33	200·98	121·98	1140·00	1018·02	231·88	67·83	517·33	200·98	121·98
Voided	287·10	215·76	46·94	33·59	91·59	43·64	71·34	293·60	220·35	48·71	36·38	92·69	42·57	73·25
Digested	852·90	802·26	184·94	34·24	425·74	157·34	50·64	846·40	797·67	183·17	31·45	424·64	158·41	48·73
Digestion coefficients (%)	74·82	78·81	79·76	50·48	82·30	78·29	41·51	74·24	78·36	78·99	46·37	82·08	78·82	40·00
***Period* 5**														
Consumed	1004·00	901·19	226·80	60·14	430·92	183·33	102·81	1004·00	901·19	226·80	60·14	430·92	183·33	102·81
Voided	262·50	200·32	45·39	29·22	82·92	42·79	62·18	258·00	195·41	44·35	30·32	81·09	39·65	62·59
Digested	741·50	700·87	181·41	30·92	348·00	140·54	40·63	746·00	705·78	182·45	29·82	349·83	143·68	40·22
Digestion coefficients (%)	73·86	77·77	79·99	51·41	80·76	76·66	39·52	74·30	78·32	80·44	49·58	81·18	78·37	39·12
***Period* 6**														
Consumed	1320·40	1179·78	273·72	77·90	616·37	211·79	140·62	1320·40	1179·78	273·72	77·90	616·37	211·79	140·62
Voided	358·60	274·98	59·28	41·10	125·04	49·56	83·62	335·80	254·37	57·09	38·99	111·92	46·37	81·43
Digested	961·80	904·80	214·44	36·80	491·33	162·23	57·00	984·60	925·41	216·63	38·91	504·45	165·42	59·19
Digestion coefficients (%)	72·84	76·70	78·34	47·24	79·71	76·60	40·53	74·57	78·44	79·14	50·00	81·84	78·11	42·09

* The care of the experimental animals was in the hands of Messrs V. Thurlbourn and C. Bendall.

† Sheep VII not included in this period.

Appendix II. *Showing nitrogen, lime and phosphate balances in sheep during digestion trials.*

Mean daily nitrogen balances.

Period	N consumed per day VII gm.	N consumed per day VIII gm.	N voided per day: Urine VII gm.	Urine VIII gm.	Faeces VII gm.	Faeces VIII gm.	Total VII gm.	Total VIII gm.	Mean daily N balance VII gm.	Mean daily N balance VIII gm.
1	—	36·29	—	25·61	—	7·09	—	32·70	—	+3·59
2	27·72	27·72	17·09	19·65	6·01	6·11	23·10	25·76	+4·62	+1·96
3	28·89	28·89	18·97	19·40	6·15	6·32	25·12	25·72	+3·77	+3·17
4	37·10	37·10	24·00	22·91	7·51	7·79	31·51	30·70	+5·59	+6·40
5	36·29	36·29	25·05	24·84	7·26	7·10	32·31	31·94	+3·98	+4·35
6	43·80	43·80	28·70	27·72	9·49	9·13	38·19	36·85	+5·61	+6·95

Mean daily lime balances.

Period	CaO consumed per day VII gm.	CaO consumed per day VIII gm.	CaO voided per day: Urine VII gm.	Urine VIII gm.	Faeces VII gm.	Faeces VIII gm.	Total VII gm.	Total VIII gm.	Mean daily CaO balance VII gm.	Mean daily CaO balance VIII gm.
1	—	14·16	—	0·24	—	12·36	—	12·60	—	+1·56
2	12·30	12·30	0·24	0·24	10·83	11·28	11·07	11·52	+1·23	+0·78
3	12·93	12·93	0·27	0·20	11·41	11·95	11·68	12·15	+1·25	+0·78
4	18·47	18·47	0·22	0·29	15·65	16·53	15·87	16·82	+2·60	+1·65
5	15·76	15·76	0·28	0·31	13·45	13·83	13·73	14·14	+2·03	+1·62
6	19·54	19·54	0·33	0·27	16·96	16·89	17·29	17·16	+2·25	+2·38

Mean daily phosphate balances.

Period	P_2O_5 consumed per day VII gm.	P_2O_5 consumed per day VIII gm.	P_2O_5 voided per day: Urine VII gm.	Urine VIII gm.	Faeces VII gm.	Faeces VIII gm.	Total VII gm.	Total VIII gm.	Mean daily P_2O_5 balance VII gm.	Mean daily P_2O_5 balance VIII gm.
1	—	10·04	—	0·04	—	9·78	—	9·82	—	+0·22
2	8·82	8·82	0·06	0·04	8·71	8·64	8·77	8·68	+0·05	+0·14
3	9·87	9·87	0·06	0·04	8·93	8·96	8·99	9·00	+0·88	+0·87
4	11·97	11·97	0·04	0·04	11·11	10·98	11·15	11·02	+0·82	+0·95
5	11·95	11·95	0·07	0·03	11·47	11·58	11·54	11·61	+0·41	+0·34
6	15·70	15·70	0·08	0·04	15·42	14·54	15·50	14·58	+0·20	+1·12

Appendix III. *Showing weight changes in sheep during digestion trials.*

	VII st.	VII lb.	VIII st.	VIII lb.
Initial weight (Apr. 20)	8	0	8	3
Final weight (Sept. 20)	9	4	9	7

PYRETHRIN I AND II.

THEIR INSECTICIDAL VALUE AND ESTIMATION IN PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM*). I.

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(With Five Text-figures.)

CONTENTS.

INTRODUCTION.

CERTAIN varieties of Pyrethrum, chiefly those derived from Persia and the Near East, have been employed for at least a century as household insecticides. The use of one of these, *Chrysanthemum cinerariaefolium*, for household and horticultural purposes has spread from Dalmatia, where it appears to have been first extensively grown, to all parts of the world; its cultivation is now widely practised particularly in Japan, France and Switzerland. Its further cultivation in temperate

[1] F. T. and R. P. H. were responsible for the chemical, C. T. G. for the entomological side of this investigation. The spray trials were done conjointly.

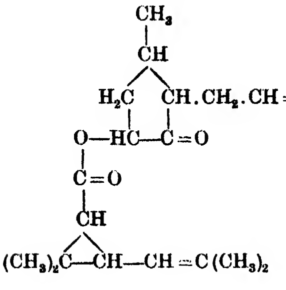
countries is limited only by the price of labour and the cost involved in harvesting the flower-heads which on account of their higher insecticidal value are usually separated from the leaf and stalk. In a paper already published (1) it has been demonstrated that *C. cinerariaefolium*, can be grown in England and that the toxicity of the English-grown flowers is equal to that of the material grown abroad. There are, however, several problems that await solution. The area under cultivation might perhaps be extended if flowers of higher poison content could be produced by systematic plant breeding, as this would lower the cost of production per toxic unit. The relationship between methods of cultivation and manuring and toxic content would also repay investigation. In this connection it has been recently suspected (3) that under continued artificial cultivation there may be a gradual lowering of the content of poison, leading to loss of value and to the necessity for more frequent replanting. Owing also to the expansion in the general use of pyrethrum, methods of rapidly standardising the product are urgently required.

In order to facilitate the study of these problems, a fairly rapid method of evaluation has become necessary. Two methods suggest themselves, (1) a direct biological determination by testing the effect produced upon suitable insects, and (2) a determination of the toxic constituents by chemical analysis. A suitable biological method for determination of insecticidal values has already been described (10) and has been extensively employed in this and other insecticide investigations. The objection to this method in the present case is that it requires considerable technical experience to employ with precision, and the harvesting of *C. cinerariaefolium* in July leaves very little time in this country for biological tests, before colder weather makes it difficult to rear suitable insects in sufficient quantity for adequate trials. A chemical method is therefore desirable.

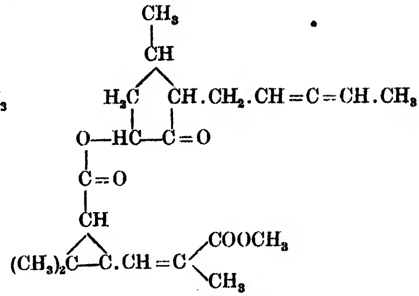
The present paper deals with a study of two methods of chemical analysis and the results obtained are as far as possible compared with a series of biological tests carried out on the same material. In order to obtain a more complete check and to investigate in detail the analytical procedure, the toxic principles have been isolated in a pure state and their insecticidal values determined.

Our knowledge of the constitution of the toxic principles of *Pyrethrum* is mainly due to the classical researches of Staudinger and Ruzicka (8 *a*) who isolated them in a pure state, established their structure, and determined the relative importance of the two constituents in contributing to the

toxicity of the flowers. They gave them the names of Pyrethrin I and II and ascribed to them the following formulae:



Pyrethrin I



Pyrethrin II

Pyrethrin I is therefore an ester of a monocarboxylic acid containing an unsaturated side-chain and a trimethylene ring, and a cyclopentalone derivative with an unsaturated side-chain (pyrethrolone). In pyrethrin II the monocarboxylic acid is replaced by the methyl ester of a dicarboxylic acid of a very similar structure.

Staudinger and Ruzicka (8b) ascribe the toxic properties of these compounds to their peculiar structure, small changes in this respect being sufficient to eliminate their insecticidal action. They showed that neither the alcoholic nor the acidic portions of the molecule were insecticidal.

Preparation of the pyrethrins.

The pyrethrins were isolated by us by the method of Staudinger and Ruzicka (*loc. cit.*). This process involves extraction with petroleum ether, a preliminary purification of the extract by means of methyl alcohol in the cold, and the removal of fatty and resin acids by shaking out a petroleum ether solution with alkali. From the oleo-resin thus obtained the pyrethrins are condensed with semicarbazide in the cold. The subsequent hydrolysis of the semicarbazone with methyl alcoholic soda splits off the acids, and the semicarbazone of pyrethrolone separates. After recrystallisation the latter is converted to pyrethrolone by mild acid hydrolysis with potassium hydrogen sulphate. The acids are separated by steam distillation, and the volatile acid (monocarboxylic) purified by distillation *in vacuo*. The dicarboxylic acid, isolated from the residue by extraction with ether, is purified by means of its crystalline chloroform compound and partially methylated. The monocarboxylic acid and the monomethyl ester of the dicarboxylic acid are then converted to the acid chlorides by the action of thionyl chloride, and after

distillation *in vacuo* condensed with pyrethrolone in the presence of quinoline to pyrethrin I and II.

Pyrethrin I was distilled *in vacuo* and boiled at 145° C. at 0·05 mm. pressure. Pyrethrin II was not distilled, as it decomposes readily at high temperatures, but was purified by solution in low boiling-point petroleum ether and subsequent cooling in a freezing mixture, which removed a small amount of resinous matter.

Toxicity tests with pyrethrins I and II.

The method employed for the determination of contact insecticidal values has already been described(10, 11) but may be briefly summarised here. *Aphis rumicis* L. (the Black Bean Aphis) has been used as the chief test insect, and large numbers are specially reared for the experiments. In order to standardise the conditions as far as possible, only insects at a certain stage of development (adult wingless females) are taken for the tests, and as the result of considerable experience, successive generations of the aphides at this stage can be obtained during the summer months, which show little individual variation in resistance as judged by the results of many duplicate experiments. Care is taken to rear the insects under uniform conditions, and they are protected from risk of attack by parasites. The spraying apparatus used (10) is designed to give percentage mortality figures under strictly controlled and constant conditions with regard to the amount of liquid sprayed, the pressure employed to produce the spray (15 lb. per sq. in.), and the length of time of contact. A large number of tests can be carried out rapidly, and by testing each compound at a number of concentrations, it is possible to draw diagrammatic curves from the results, plotting percentage mortality against concentration, and thus to obtain a convenient means of comparing toxicities.

In making up the mixtures for spraying, small amounts of the pyrethrins were weighed out, dissolved in a little alcohol in a graduated flask and diluted with a 0·5 per cent. aqueous solution of saponin to assist spreading and wetting, both compounds being thus obtained at known concentrations and in a very finely distributed condition. Dilutions were made with 0·5 per cent. saponin solution. Control experiments, many times repeated, have shown that 0·5 per cent. or 1·0 per cent. solutions of saponin are without toxicity to *A. rumicis*.

After spraying, the insects were placed within reach of fresh bean foliage and were examined and classified on the two following days. At each examination counts were made under four headings: "un-

affected," "slightly affected," "moribund," and "apparently dead," and the results are recorded thus in Table I.

Table I. *Toxicities of Pyrethrin I and II to* A. rumicis.

(N = not affected, S = slightly affected, M = moribund, D = apparently dead.)
Marks allowed for each concentration = ($\frac{1}{4}$S % + $\frac{1}{2}$M % + D %).

	No. of tests	Concentration in gm./100 c.c.	N %	S %	M %	D %	M and D %	Marks
Pyrethrin I (not distilled)	2	0·05	—	—	—	100	100	100
	2	0·025	—	—	—	100	100	100
	2	0·01	—	—	—	100	100	100
	3	0·005	—	—	—	100	100	100
	3	0·0025	—	—	27	73	100	86·5
	2	0·001	5	10	70	15	85	52·5
	2	0·0005	55	25	10	10	20	21
	1	0·00025	70	30	—	—	0	7·5
Pyrethrin I (after distillation *in vacuo*)	1	0·05	—	—	—	100	100	100
	1	0·025	—	—	—	100	100	100
	1	0·01	—	—	—	100	100	100
	2	0·005	—	—	20	80	100	90
	2	0·0025	—	—	25	75	100	87·5
	1	0·001	—	30	70	—	70	42·5
	1	0·0005	70	20	—	10	10	15
Pyrethrin II	1	0·05	—	—	50	50	100	75
	3	0·025	—	7	50	43	93	70
	3	0·01	10	6·5	67	16·5	83·5	51·5
	4	0·005	30	22·5	27·5	20	47·5	39
	3	0·0025	53·5	20	—	26·5	26·5	31·5

Pyrethrolone	Not toxic at a concentration of 0·2 gm. per 100 c.c.
The monocarboxylic acid	,, ,, ,, ,,
The dicarboxylic acid ...	,, ,, ,, ,,

For purposes of diagrammatic representation we have used two methods of evaluating these classes. In the first method (*a*) we have expressed the moribund and dead together as a percentage of the total number of insects sprayed at each concentration. In the second method (*b*) we have awarded at each concentration arbitrary valuations or marks by adding to the percentage of dead half the percentage of moribund and a quarter of the percentage of the slightly affected. This method of treatment may be a slight understatement of the values at the higher concentrations. In both methods of computation, the values at the lowest concentrations are only approximate. Those values ranging between 90–40 per cent. are of greatest importance for purposes of comparison.

The results are shown in Diagrams 1 *a* and 1 *b*, in which the values given by both methods of computation are plotted against the concentrations in grammes per 100 c.c. In the case of pyrethrin I, the scale is

ten times that used for pyrethrin II, this procedure enabling the results to be expressed in an area of reasonable dimensions.

An inspection of Table I and Diagrams 1 *a* and 1 *b*, shows that Pyrethrin I is many times more toxic than pyrethrin II, to *Aphis rumicis*.

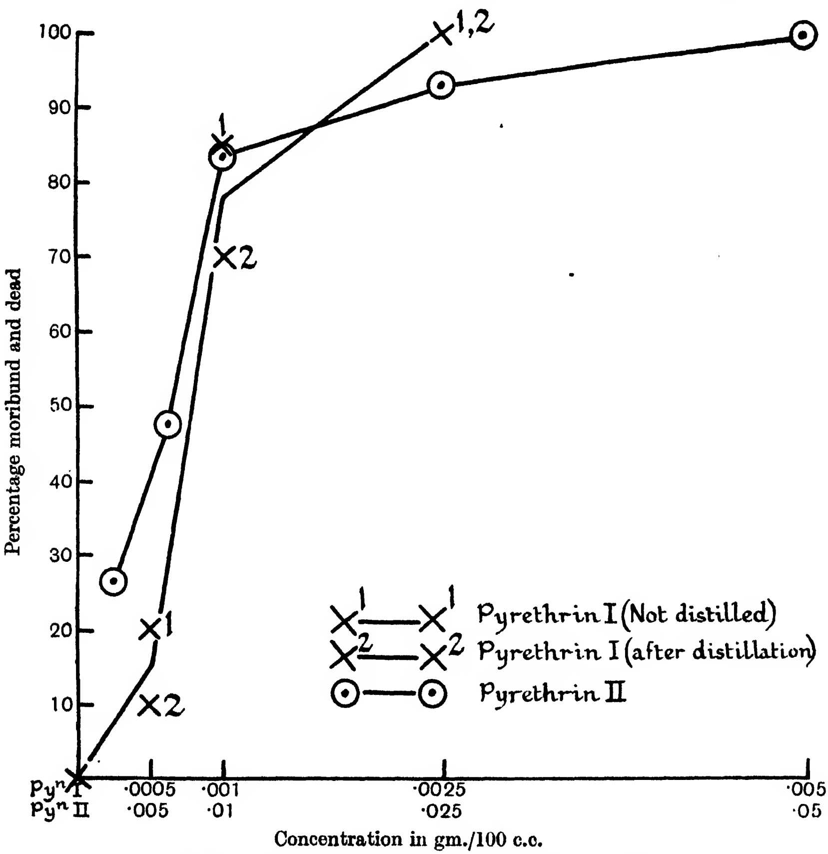


Diagram 1 *a*. Toxicities of pyrethrin I and II to *A. rumicis*. Toxicities evaluated in terms of percentage of moribund, and dead insects.

Staudinger and Ruzicka (8*c*) found that to cockroaches pyrethrin I was the more toxic and they express the opinion that the insecticidal effect of pyrethrum is almost wholly due to pyrethrin I.

In view of the method of synthesising pyrethrin II, from pyrethrolone and the dicarboxylic acid, there is the possibility that a certain amount of an isomer may be present in the product, which may result in a loss of toxicity. Later work on extracts of the flower-heads to be described

below, indicates, however, that except at higher concentrations, the toxicity of the pyrethrum can be closely correlated with its content of pyrethrin I, thus confirming the lower toxicity of pyrethrin II.

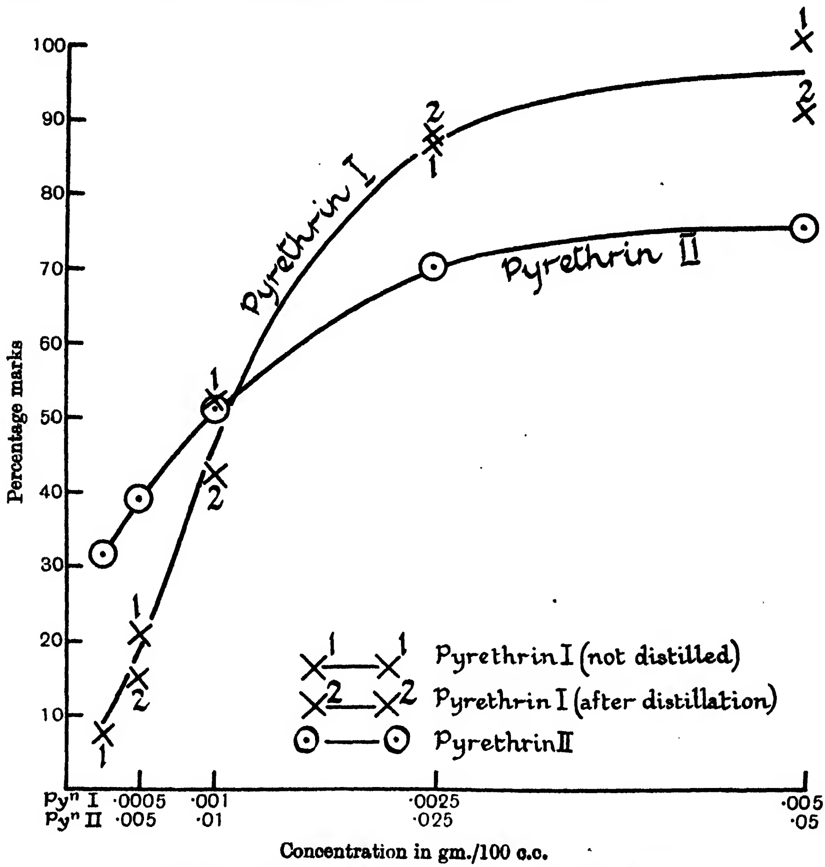


Diagram 1 *b*. Toxicities of pyrethrin I and II to *A. rumicis*.
Toxicities evaluated by marks.

Pyrethrin I possesses a higher insecticidal value by contact than any compound tested by us; the only substance which in our experience approaches it in toxicity is rotenone (tubatoxin), the characteristic constituent of the fish-poison, *Derris* (*Deguelia elliptica*) and of White and Black Haiari (*Lonchocarpus* spp.). Calculated from the amount of spray falling on an individual aphis of the size used in our experiments, the lethal dose is approximately 3×10^{-6} to 6×10^{-6} gm. of pyrethrin I, and of this only a small fraction comes into effect.

Pyrethrin II is of a lower order of toxicity than pyrethrin I, but none the less is an exceedingly toxic substance, ranking higher than nicotine in this respect.

The constituent parts of the pyrethrins were not found toxic at any concentrations tested, pyrethrolone, and the mono- and dicarboxylic acids having negligible effects. The toxicity of both compounds is, therefore, due to some critical make-up of the pyrethrin molecule taken as a whole.

METHODS OF ESTIMATION OF THE PYRETHRINS.

The drawbacks to the biological method of evaluating pyrethrum, lie in the facts that it is somewhat difficult to obtain a satisfactory supply of suitable insects throughout the year, that it requires biological equipment for breeding and rearing insects in a condition suitable for the work, and that the technique requires time and special training for its proper application. Although a biological method will probably remain the final court of appeal in evaluating such an insecticide, it is nevertheless true that a chemical method, provided it is sufficiently rapid and accurate, is urgently needed. For plant-breeding purposes it should prove invaluable, if from time to time it were backed up by direct tests on insects. The biological method used by us only calls for small quantities of material, which is also a *sine qua non* in the case of an analytical process applied to purposes of plant breeding.

Staudinger and Harder (9) have published an account of two chemical methods, which may be described as (*a*) the acid, and (*b*) the semicarbazone method. We have undertaken a critical study of both with the object of making them shorter and of reducing the amount of material required for the analyses.

The acid method.

Staudinger and Harder (9) extracted 500 gm. of the powder with low-boiling petroleum ether, and after evaporating off the solvent extracted the residue with methyl alcohol. Ten per cent. of water was added, and after cooling in a freezing mixture the precipitate of resins and fats was filtered off. The filtrate was then hydrolysed with an excess of methyl alcoholic soda, the methyl alcohol taken off in partial vacuum and the residue extracted with ether. The mixture was then acidified and distilled in steam for two hours, the distillate being titrated with $N/10$ soda. Harder (2), however, recommends the separation of the monocarboxylic acid from the distillate by extraction with petroleum ether, and titrating after evaporating the volatile solvent. From the value for the acid the pyrethrin I content was calculated.

Pyrethrin II was determined in the residue after steam distillation, which after clearing with animal charcoal was extracted with methylated ether in a Kutscher-Steudel apparatus, the extract being afterwards evaporated and titrated. This gave a value for the dicarboxylic acid from which the pyrethrin II content was calculated.

We have attempted to adapt this method for use with very much smaller quantities of material, and for each analysis have used 10 gm. of flowers or 50–100 gm. of stalk.

For an examination of each stage of the analysis an exceptionally toxic sample of the flowers was chosen. Four lots of 10 gm. were completely extracted with petroleum ether and the solvent was taken down in a strong current of CO_2, the last traces being eliminated by evacuation in a desiccator. The residues were subjected to the following treatments:

1. The extracts obtained by gentle warming with four lots of 2·5 c.c. of methyl alcohol (purified over caustic soda), were collected in a centrifuge tube, cooled in ice and salt, and centrifuged clear; 10 per cent. of water by volume was added and after cooling in ice and salt, the extract was again centrifuged. The supernatant liquid was poured off from the residue into a 100 c.c. long-necked flask, and the washings of the residue added (two lots of 2 c.c. of alcohol containing 10 per cent. of water). The pyrethrins were estimated by saponification and determination of the volatile and water-soluble acids.

2. Extracted with four lots of 2·5 c.c. of methyl alcohol containing 10 per cent. of water, cooled in ice and salt one hour and centrifuged clear. Treatment then as 1.

3. As 2, but cooling in ice omitted.

4. Extracted with absolute methyl alcohol, cooled under the tap and filtered through a small wad of fat-free cotton wool. Hydrolysis and the remainder of the treatment was afterwards as in 1.

The results were as follows:

Pyrethrin I (%)	(1) 0·57	(2) 0·56	(3) 0·56	(4) 0·59
,, II (%)	0·47	0·49	0·49	0·57

The total residues from 1 were examined further by extraction with alcohol containing 10 per cent. of water and found to contain 0·02 per cent. pyrethrin I and 0·04 per cent. pyrethrin II.

The values for pyrethrin I are in substantial concordance and, although that of pyrethrin II in (4) is higher than in the other cases, there is obviously some risk of loss by adsorption by the flocculent pre-

cipitates produced on adding water to the methyl alcohol extracts. As, moreover, pyrethrin II contributes only in a minor degree to the toxicity of pyrethrum, we have preferred to use pure methyl alcohol for extraction and have omitted the addition of water. This procedure obviates the risk of loss by frothing when the methyl alcohol is taken off *in vacuo*, and shortens and simplifies the process materially.

Tests were made on various samples, to ascertain the effect of omitting the extraction with methylated ether after hydrolysis, with the following results:

Origin of sample Flowers		Extracted Py. I %	Extracted Py. II %	Not extracted Py. I %	Not extracted Py. II %
Swanley ...	(1927)	0·41	0·47	0·41	0·40
Wye	(1927)	0·37	0·34	0·35	0·32
Harpenden ...	(1928)	0·59	0·67	0·59	0·73
		0·58	0·70		
Harpenden stalk	(1928)	0·028	0·033	0·031	0·034

The differences between the results are small and within the experimental error, and although most of our tabulated results have been obtained after methylated ether extraction after saponification, this process does not appear to serve any very useful purpose.

The determination of the monocarboxylic acid and pyrethrin I. On distilling in steam a sample of pyrethrum extract treated as above, the distillate is acid even after prolonged distillation; one sample (10 gm.) gave the following figures in c.c. for the titration with $N/50$ soda for each 20 c.c. of distillate:

(1) 5·72, (2) 1·6, (3) 1·15, (4) 0·64, (5) 0·51, (6) 0·37.

When an amount of the pure acid equivalent to 10 gm. of a rich sample of pyrethrum is distilled in the same way, 95 per cent. comes over in the first 20 c.c. and practically the whole is recovered in 40 c.c. of distillate. In the case of pyrethrum extracts, therefore, only a portion of the acid in the distillate is the monocarboxylic acid.

It was considered by us for some time that examination of the distillation curve would yield some method of differentiating between the acid associated with pyrethrin I and the other volatile acids in the distillate, but it was found, particularly with a micro-method, that the process could not be controlled with sufficient accuracy to yield data of value on this point. We have therefore employed extraction with low boiling petroleum ether to effect a separation of the monocarboxylic acid. We have found that the pure acid in an amount equivalent to that present in our determinations, distributes itself between petroleum

ether and water in the ratio of 15 to 1. The repeated extraction of one of the distillates from pyrethrum with an equal volume of petroleum ether gave the following titration figures after evaporation of the solvent:

1st extract	required	8·8 c.c.	$N/50$ soda.
2nd	"	0·4	"
3rd	"	0·1	"
4th	"	0·1	"

These figures show that this method separates the monocarboxylic acid quantitatively, and we have therefore extracted the first 50 c.c. of the distillate twice with an equal volume of petroleum ether and subtracted a blank of 0·2 c.c., as 0·1 c.c. appears to be a constant blank for each extraction. Relatively large amounts of acid insoluble in petroleum ether are present in the distillate; the values obtained with a number of samples of pyrethrum flowers when the distillates were extracted with petroleum ether and titrated with $N/50$ caustic soda gave the following results in c.c.:

Acid soluble in petroleum ether	(1) 6·25	(2) 5·78	(3) 5·96	(4) 6·86
Acid insoluble in petroleum ether	8·95	8·8	6·75	7·2

It is therefore evident that there is no obvious relationship between the titration value of the gross distillate and the amount of the monocarboxylic acid present.

The determination of the dicarboxylic acid and pyrethrin II. The dicarboxylic acid is left in solution in the distillation flask. We have shown with the pure acid that prolonged distillation in steam does not lead to any loss. It has therefore been our practice after collecting the first 50 c.c. containing the monocarboxylic acid to continue the steam distillation for another 100 c.c.; this leads to an aggregation of the resinous particles and facilitates subsequent filtration. Staudinger and Harder used animal charcoal followed by filtration for the purpose of clearing the solution. Our experience has been that this leads to loss of the dicarboxylic acid, and if a sufficient quantity of charcoal is used to its complete adsorption. We have therefore added, instead, 0·2 gm. of pure calcium sulphate to the hot liquid in the flask, which is allowed to stand overnight to cool. The calcium sulphate crystallises on cooling, and clears the liquid. We have shown that calcium sulphate neither adsorbs nor combines with the acid. After this treatment the filtered solution is extracted with methylated ether and the dicarboxylic acid in the extract titrated with $N/50$ soda.

For the extraction with methylated ether we have used an automatic

extractor. The apparatus, which is shown in Fig. 1[1], presents little difficulty in making; it is only necessary to mention that the internal tube has a small pellet of glass sealed on at the bottom to prevent the

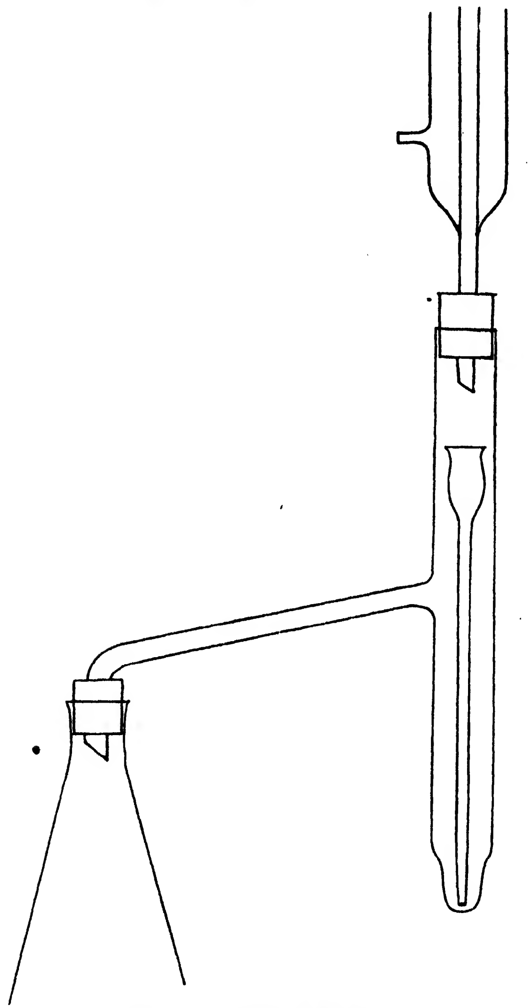


Fig 1. Automatic extractor.

stoppage of the flow of ether, that the hole at the bottom should be constricted to give a fine stream of bubbles, but should not be so small as to become clogged by the deposition of calcium sulphate which takes

[1] The scale of the figure is from one quarter to one fifth actual size.

place as the solution becomes saturated with ether. The ether is boiled in the conical flask and on condensing flows through the inner tube and returns after percolating the aqueous layer.

Description of acid method. The procedure finally adopted in the acid method is therefore as follows:

Ten gm. of the ground flower-heads or 50 gm. of the stalk are extracted by low boiling-point petroleum ether (B.D.H. A.R., boiling range below 40° C.) in a Soxhlet extractor heated over a carbon filament lamp; when extraction is complete, the petroleum ether is evaporated to a small bulk in a rapid current of CO_2 with very gentle warming, the evaporation being completed in a vacuum desiccator. The residue is extracted successively with four (or in the case of the stalk, six) lots of 2·5 c.c. of absolute methyl alcohol[1] (free from acid) with gentle warming on a water-bath; each extraction is cooled under the tap, filtered through a small wad of fat-free cotton wool into a long-necked flask of 100 c.c. capacity, the extraction flask being finally rinsed with 2·5 c.c. of cold methyl alcohol. The clear solution is treated with 4 c.c. of $N/1$ caustic soda in methyl alcohol and boiled under a reflux condenser for six to eight hours after which the methyl alcohol is taken off in partial vacuum with gentle warming; a right-angled tube connection to the condenser is used to stop the spray from being carried over. The temperature should not be allowed to rise so high that the alcohol condenses in the connecting tube. The stopper and connecting tube are washed down into the flask, a little water is added to bring the soaps into solution, 6 c.c. of $N/1$ sulphuric acid is added, and the acid liquid distilled in steam. The distillation apparatus used by us was similar to the original Pregl micro-Kjeldahl still (6), except that the rubber connections with the condenser are eliminated so that the glass tube connects directly with a small worm condenser and leads to a receiver marked at the 50 c.c. level. 50 c.c. of distillate are collected for the determination of the volatile acid, and the distillation then continued until a further 100 c.c. have been collected. The first 50 c.c. of distillate is then transferred to a pear-shaped funnel closed with a rubber stopper and is extracted twice with 50 c.c. of low boiling-point petroleum ether which can be conveniently measured out in the receiver. For complete extraction vigorous shaking is necessary, each extract being washed with a small amount of distilled water. After adding 20 c.c. of water to the extract the ether is gently evaporated and the residue titrated with $N/50$ caustic soda using

[1] A high-grade commercial methyl alcohol free from acetone was boiled under reflux with caustic soda for several hours and fractionated.

phenolphthalein. As the acid tends to adhere to the glass it is necessary to wash down the sides of the flask with neutralised alcohol. We have titrated to a distinct pink which after shaking remained for at least one minute[1].

To the hot residue in the flask, which should not exceed 40 c.c., 0·2 gm. of pure calcium sulphate is added and after standing overnight the solution is filtered into the automatic extractor, already described, through a small wad of fat-free cotton wool, just tight enough to give a gentle flow. The filtrate should be crystal clear. The extraction is carried out with acid-free methylated ether for a period of eight hours, after which 20 c.c. of distilled water are added to the ether extract and the ether gently taken off; the aqueous layer is finally filtered through a loosely packed wad of fat-free cotton wool and the dicarboxylic acid titrated with $N/50$ soda.

The factors to be used are as follows:

$$\begin{aligned} 1 \text{ c.c. } N/50 \text{ alkali} &= \tfrac{168}{50} = 3\cdot36 \text{ mg. monocarboxylic acid,} \\ &= \tfrac{330}{50} = 6\cdot6 \text{ mg. pyrethrin I,} \\ &= \tfrac{99}{50} = 1\cdot98 \text{ mg. dicarboxylic acid,} \\ &= \tfrac{187}{50} = 3\cdot74 \text{ mg. pyrethrin II.} \end{aligned}$$

The semicarbazone method.

Whereas the acid method has presented no great difficulty in adapting to a small scale technique, the determination of the pyrethrin content by means of the semicarbazone required considerable modification. Moreover, the procedure is tedious and gives only an approximate estimation of the sum of the two pyrethrins, and as pyrethrin I is the main toxic constituent of pyrethrum, there is some objection to its use for practical purposes; we have, therefore, only used it to confirm that the amounts of the acids are a true measure of the pyrethrin content.

The procedure adopted by Staudinger and Harder (*loc. cit.*) for the semicarbazone method was as follows: They used 500 gm. of the powder and carried out the extraction with low boiling-point petroleum ether and the purification with 90 per cent. methyl alcohol exactly as in the acid method. The oil obtained after evaporation of the solvent was

[1] Further work, to be published separately, has shown that for routine analyses a very rapid assay of pyrethrin I can be made by extracting the plant material with petroleum ether (boiling range 40–50° C.), transferring the petroleum ether solution to the flask used for distilling, hydrolysing with $N/1$ soda in methyl alcohol for one to two hours, acidifying and distilling in steam until two lots of 50 c.c. of aqueous distillate have collected. The petroleum ether distilling is used for the first extraction of monocarboxylic acid, which is titrated without evaporating the solvent.

dissolved in methyl alcohol and treated with an excess of semicarbazide hydrochloride and sodium acetate in solution in a small amount of water. After standing 24 hours the whole was evaporated *in vacuo* to dryness and the residue was washed with water to remove the uncombined semicarbazide. The semicarbazone was then estimated by a determination of the nitrogen in the residue by Wilfarth's (12) modification of the Kjeldahl method, in which a mixture of fuming sulphuric and phosphorus pentoxide is used with mercury as a catalyst; the amount of the pyrethrin was then calculated.

Determination of semicarbazone. The use of the Kjeldahl method for estimating semicarbazones is open to criticism, as no modification of this method, to our knowledge, ammonifies quantitatively the hydrazo grouping. We have found that pure semicarbazide hydrochloride reacts to the Kjeldahl method to the extent of about 30–40 per cent., but it is probable that mixtures containing other organic compounds (such as the impure pyrethrin semicarbazones) would react more completely, as it is well known that the presence of oxidisable carbonaceous material effects a partial reduction of such nitrogen groupings to ammonia; nevertheless it is apparently quite impossible by the addition of reducing agents to convert hydrazo compounds to ammonia quantitatively. We have therefore abandoned the Kjeldahl method for this purpose.

We have worked out a method suitable for the micro-analysis of semicarbazones by combining and modifying the methods of Rimini (7) and of Maselli (4). The essential reactions are as follows:

$$(1)\ NH_2.NH.CO.NH_2 + H_2O = NH_3 + CO_2 + NH_2.NH_2,$$
$$(2)\ NH_2.NH_2 + 2HgCl_2 = N_2 + 4HCl + 2Hg.$$

Rimini hydrolyses semicarbazide and semicarbazones with acid (reaction (1)) and then oxidises the hydrazine with mercuric chloride in alkaline solution (reaction (2)) measuring the nitrogen evolved. Maselli carries out acid hydrolysis and determines either the hydrazine by titration with potassium iodate or the ammonia by making alkaline and distilling. We have carried out the reactions (1) and (2) in one stage by boiling with a hydrochloric acid solution containing mercuric chloride; the semicarbazide is broken down into hydrazine (which is then oxidised to nitrogen) and ammonia, which can be determined readily even in very small amounts. The presence of the mercuric chloride is necessary in the case of semicarbazones in order to oxidise the hydrazine and so prevent its reduction by the ketone formed or by any organic impurities. This reduction was found to occur if semicarbazide hydrochloride was

boiled with hydrochloric acid to which sucrose had been added, as more than one-third of the total nitrogen was found as ammonia.

The time required for the complete hydrolysis of semicarbazide by 15 per cent. hydrochloric acid containing 5 per cent. mercuric chloride is six hours, as the following results with the pure hydrochloric acid show:

After $1\frac{1}{2}$ hours	hydrolysis	28·3 %	of total nitrogen recovered as NH_3
,, 4	,,	32·8	,, ,, ,,
,, 6	,,	33·25	,, ,, ,,

In the case of pyrethrin semicarbazones seven hours hydrolysis is therefore allowed.

We have shown that a pyrethrum extract does not interfere with the reaction, by hydrolysing a known amount of semicarbazide to which had been added an approximately equivalent amount of pyrethrum extract; the amount of ammonia found corresponded to 34 per cent. of the total nitrogen of the semicarbazide. The small increase over the theoretical value of 33·3 per cent. is probably due to traces of ammonia produced from the extract.

Conversion of pyrethrins to semicarbazones. Apart from the question of determining the amount of semicarbazone, the adaptation of the method of Staudinger and Harder as a micro-determination presents two main difficulties, (1) the complete condensation of minute amounts of ketone with semicarbazide, and (2) the removal of this reagent from the product.

(1) We have examined the conditions necessary for complete condensation. Ten gm. of flower-heads were extracted with low boiling-point petroleum ether; after removing the solvent *in vacuo* the residue was extracted with methyl alcohol and the solution filtered and made up to 10 c.c. For each analysis 4 c.c. (equivalent to 4 gm. of powder) were taken. We have omitted the preliminary precipitation of the methyl alcohol solution with 10 per cent. of water, as extracts purified in this way gave low results. The lower figures are due to the presence of the water and not to the removal of impurities that interfere, for if the water was removed *in vacuo* and the oil taken up in pure methyl alcohol before condensing the same results were obtained as with untreated methyl alcohol extracts. At 25° C. the reaction with semicarbazide appears to be complete in 36 hours, provided the methyl alcohol solution is first concentrated to a volume of $\frac{1}{2}$–1 c.c. The results reach a maximum after 36 hours and then remain constant within the

experimental error; this is shown by the following figures for one sample:

24 hours at 25°		0·80 %	pyrethrins.
36	,,	1·24	,,
48	,,	1·09	,,
72	,,	1·17	,,

Four days standing at room temperature gave approximately the same results, but a longer period gave higher figures. We believe this to be due to resinification which renders the subsequent removal of the excess of semicarbazide very difficult.

(2) After condensation, it is necessary to evaporate off the alcohol as the semicarbazone does not crystallise out and the addition of water produces an emulsion which cannot be separated. In spite of the small amount of material we have found great difficulty in washing out the free semicarbazide from this residue, even when it is spread out in a very fine film. In order to do this we have found it necessary after each washing to take up the residue in ether and evaporate down again to a film. A dilute solution of acetic acid and sodium acetate was used for washing. The semicarbazide in the washings was then titrated with potassium permanganate, and it was found necessary to repeat the process of solution, evaporation, and washing, three times; further repetition continued to give a small constant titration figure in the washings but this was found to be due to some soluble organic substance, as a pyrethrum extract which had not been treated with semicarbazide gave a similar result. We have also used an alternative method by taking up the residue (after evaporating off the alcohol) with ether and washing the ether solution with water in a separating funnel. As part of the semicarbazone does not dissolve in ether it is necessary to filter the aqueous washings. Similar results were obtained by the two methods as is shown by the following results of three analyses of the same sample.

Washed 3 times as a film in the tube 1·00 % pyrethrins.
Washed 6 times as a film in the tube 1·10 % pyrethrins.
Ether solution washed in a separating funnel 1·02 % pyrethrins.

Description of the semicarbazone method. Our procedure in the semicarbazone method is then as follows: 10 gm. of the powdered flower-heads are extracted completely with low boiling-point petroleum ether and the residue, after evaporation of the solvent, is extracted with absolute methyl alcohol, in exactly the same way as for the acid method.

The extracts are filtered successively into a small measuring cylinder and brought to a volume of 10 c.c. The solution is mixed and 4 c.c. aliquots are pipetted into a hard glass test-tube. 30 mg. of semicarbazide hydrochloride and 50 mg. of sodium acetate are added and the solution is concentrated to about 0·5 c.c. This is done by inserting a rubber stopper having a capillary tube reaching to the bottom of the liquid and a side-tube which is connected to a pump; the test-tube is kept at a temperature of 25° C. and the alcohol can then be readily removed by evacuation. A small glass bead, to act as a spray-trap, was inserted in the side-tube and held in place by constricting the tube on either side. At the finish a few drops of methyl alcohol are poured into the side tube, to free it from any deposit carried up by the spray, and so manipulated as to wash down the capillary as well. The test-tube is corked and placed in a bath at 25° for 36 to 48 hours. The methyl alcohol is then evaporated to dryness by connecting to a pump and evacuating without the use of a capillary. Two methods of washing have been employed. (*a*) A little ether is added to the residue and the solution is then evaporated to a film by tilting and rotating the test-tube which is prevented from cooling by warming with the hand. The last traces of ether are removed in a good vacuum. The film should cover half the length of the test-tube. Twenty c.c. of a solution containing 0·1 per cent. acetic acid and 1 per cent. sodium acetate are poured carefully into the tube and left to stand 10 minutes; the film should be completely covered. The solution is then filtered through a small plug of cotton wool and the test-tube rinsed out with about 2 c.c. of water. The film is then remade by solution in ether and evaporation and is again washed, the whole process being carried out three times in all. The small residue on the filter is returned to the test-tube by pouring through two drops of methyl alcohol and 1 c.c. of ether. The alcohol and ether are evaporated *in vacuo*. Five c.c. of a 15 per cent. solution of hydrochloric acid containing 5 per cent. of mercuric chloride are heated and poured through the filter into the test-tube. (*b*) The alternative and more rapid method of washing is as follows: after complete removal of the methyl alcohol, the residue is twice extracted with 5 c.c. of ether and the ether solution poured into a small separating funnel. The residue in the test-tube is washed four times with 10 c.c. of water, the washings are successively filtered through a cotton wool plug into the separating funnel and used to wash the ether solution with gentle rotation. The ether solution is washed twice more with small amounts of water, returned to the test-tube and evaporated. Five c.c. of the 15 per cent. hydrochloric acid solution

containing 5 per cent. mercuric chloride are heated and used to wash into the test-tube the insoluble material left in the separating funnel and the filter, the cotton-wool plug in the latter being previously loosened.

The test-tube is fixed to a small reflux condenser and after the addition of a little pumice the solution boiled for seven hours. Ammonia is then determined by the method adopted by Pregl in his revised micro-Kjeldahl method (6).

The solution used for rendering alkaline was prepared by diluting a 40 per cent. solution of caustic soda with an equal volume of a saturated solution of sodium thiosulphate which is necessary for the decomposition of the mercury ammonium complex. $N/50$ acid was used for absorbing the ammonia, and $N/50$ alkali for titration.

For the mixed pyrethrins which are present in nearly equal proportions we have taken 350 as the approximate mean molecular weight, 1 c.c. of $N/50$ acid being then equivalent to 70 mg. of the combined pyrethrins.

Results of analysis.

In Table II the results obtained by the acid method and also in certain cases by the semicarbazone method are given in duplicate. The results give some idea of the agreement to be obtained by parallel determinations by the acid method. In addition, the table shows that there is reasonable agreement between the sum of the percentages of pyrethrin I and II as obtained by the acid method and this value as determined by the semicarbazone method.

The correlation between the contents of pyrethrin I and II. The data in Table II indicate that there is an apparent connection between the values for the two pyrethrins. We are indebted to Dr Wishart of the Rothamsted Statistical Dept. for kindly analysing the figures for the first eight samples in the table. He finds the correlation coefficient between pyrethrin I and II in these samples to be positive and to have a value of 0·85. This is definitely significant and although the number of samples is small and the seed from which they were grown was of the same origin, there is a distinct suggestion of a close connection in the derivation of the two poisons in the plant, either from some parent body or by some chemical reaction common to the synthesis of both or it may be that one compound is derived from the other.

The relationship between size of flower-heads and the content of the pyrethrins. For a long time there has been an opinion that the closed

and half-closed flowers of pyrethrum are more toxic to insects than fully opened ones, although critical examination has thrown doubt on this view.

Table II. *Results of analysis of various samples of pyrethrum.*

Origin of sample	Acid method: Pyrethrin I	Acid method: Pyrethrin II	Acid method: Total	Semi-carbazone method	Weight of 100 flower-heads gm.	Standard deviation
		1927 Harvest				
Harpenden	0·35	0·36	0·71	0·71	13·91	0·27
	0·36	0·35	0·71	0·74		
Reading	0·39	0·33	0·72	—	11·65	0·31
Scilly Isles	0·43	0·46	0·89	—	12·87	0·31
	0·46	0·46	0·92			
Seale Hayne	0·28	0·31	0·59	0·60	13·95	0·44
				0·58		
Swanley	0·41	0·46	0·87	0·90	9·62	0·26
	0·41	0·48	0·89	0·86		
Wisley	0·39	0·42	0·81	0·82	11·22	0·29
	0·39	0·41	0·80	0·86		
Worcester	0·46	0·57	1·03	1·10	9·59	0·29
	0·45	0·54	0·99	1·00		
Wye	0·35	0·32	0·67	0·74	9·66	0·16
	0·38	0·33	0·71	0·71		
		1926 Harvest				
Scilly Isles	0·60	0·49	1·09	1·13*		
	0·59	0·58	1·17			
Wye	0·37	0·34	0·71	0·77		

Origin of sample	Acid method: Pyrethrin I	Acid method: Pyrethrin II	Acid method: Total	Semi-carbazone method	Petr. ether extract %	Pyrethrin I and II % of petr. ether extract
		1928 Harvest				
Harpenden grown						
Plot 20 *c*						
Flowers	0·58	0·70	1·28	1·18	5·44	23·6
	0·59	0·70	1·29	1·14		
Flowers and Stalk	0·25	0·32	0·57	—	2·95	20·0
	0·26	0·35	0·61			
Stalk	0·031	0·034	0·065	—	1·13	5·6
	0·028	0·033	0·061			
Plot 17 *c*						
Flowers	0·47	0·59	1·06	—	5·75	18·6
	0·47	0·61	1·08			
Flowers and stalk	0·23	0·31	0·54	—	3·36	16·0
Stalk	0·032	0·036	0·07	0·09	1·07	6·5

* Mean of six determinations.

The data in Table II for the first eight samples were used for testing the dependence, if any, of the pyrethrin values upon the size of flower-heads. Random samples were taken in a suitable way and the weight

of ten lots of ten heads of each sample determined, giving the weight of each hundred heads. The standard deviations of the mean, calculated from these figures, show a certain amount of variation between the samples in point of size and uniformity. The data were subjected to statistical analysis by Dr Wishart, who found the correlation between the weight per 100 flower-heads and percentages of pyrethrin I to be $-0{\cdot}527$ and that for pyrethrin II $-0{\cdot}543$. The correlation while reasonably large and negative cannot be regarded as significant for so small a number as eight samples.

The data are too meagre to settle the matter finally, but taking individual samples and comparing them with each other we find how uncertain any choice resting upon a selection by size of flower-heads would be and that the source of origin is at least as important. For example, although in the Worcester and Seale Hayne samples, the ratio of the pyrethrin contents is as 1·0 to 0·6 and the weight per 100 heads as 9·6 to 14 respectively, in the case of the Wye and Harpenden samples, whereas the proportionality in the weight of heads is the same (9·6 to 14), that of the pyrethrin contents is as 0·7 to 0·7. Moreover, the loss of yield, ensuing from harvesting the smaller-sized heads of the closed and half-closed flowers as against taking the crop when they are fully open, outweighs in economic importance any advantage likely to accrue from the possibility of the former possessing a larger amount of the two pyrethrins.

The loss on heating at 105° C. was determined, but, as this did not vary amongst the samples to an extent sufficient to modify the deductions drawn from the analytical results, we have preferred to express our data on the air-dried samples.

The pyrethrin content of flowers, stalk, and mixed stalk and flowers. Table II gives data for the pyrethrin content of two samples of stalk, in addition to those of the flowers and the mixed flowers and stalk from two beds, 17 *c* and 20 *c* at Harpenden; in addition, it contains the percentage amounts of the petroleum ether extracts and also their content of pyrethrin. These samples were carefully taken and, after air-drying in the shade, each was divided into three portions; in one the proportion of flower-heads to stalk was determined, the stalk and flowers being ground separately; the second portion was ground as a whole, while a third portion was kept for reference. The pyrethrin content of the stalk is between one-fifteenth and one-twentieth of that of the flowers. Although we have no exact data, the few tests carried out biologically indicated a similar relationship in the toxicity of the stalk and flowers

respectively, and experiments carried out in previous years on the plants from these beds indicated that the stalk is in general less than one-tenth as toxic as the flower-heads.

The percentage weights of petroleum ether extracts of the flowers, of the mixture of flowers and stalk and of the stalk are very different, the stalk for both beds 17 *c* and 20 *c* giving only about one-fifth of that of the flowers. The contents of pyrethrin were therefore calculated on these extracts and on that of the mixed stalk and flower. The counterbalancing effect of the small weight of extract from the stalk upon the percentage of pyrethrins is considerable and as a result an extract of the mixture of stalk and flower contains in the case of plot 17 *c* 16·6 per cent. pyrethrin against 18·6 per cent. in the flowers, and in the case of 20 *c* 20·0 per cent. against 23·6 per cent. In the case of the mixture the total amounts of pyrethrin extracted per unit weight of plant material is of course about one-half that of the flowers.

The results of the analyses of the flowers, the stalk and a mixture of the two afford a means of testing the validity of the method for use with material containing both stalk and flowers. In the sample 20 *c* the mixture of flowers and stalk contained approximately 44 per cent. of flowers; a calculation from the percentages of pyrethrin I gave 40·5 per cent. and from pyrethrin II 45 per cent. The mixture from bed 17 *c* contained 46 per cent. of flowers; the analysis gave 46 per cent. calculated from pyrethrin I and 49 per cent. from pyrethrin II. The presence of stalk in the sample did not, therefore, introduce any complicating factor in the analysis.

COMPARISON OF ANALYTICAL RESULTS WITH TOXICITY TESTS.

The final criterion of the analytical method lies in the degree of concordance with the results of toxicity tests with insects. Quantitative investigations with biological material are by the nature of the case less accurate than chemical analyses, and the determination of results depends largely on the quality of the biological material, which may fluctuate, and upon the care taken in observing the effects produced. In order, therefore, that there should be entire freedom from bias, the two sides of this investigation were kept entirely independent of each other and no comparison was made between the results until both were complete. The biological trials were carried out in the following way: 10 gm. of each sample of ground flowers were allowed to soak in a known amount of absolute alcohol for some days, dilutions were then made by pipetting off the clear supernatant solution

into 0·5 per cent. solutions of saponin in water. Control tests showed that the insects used could tolerate much higher proportions of alcohol and saponin than were used in these experiments. The insects were then sprayed in the manner already described and a short time later duplicate experiments were carried out. As in the case of the biological tests with the pyrethrins which were made within a few days of these experiments, an observation of the results was made after 24 and again after 48 hours. The toxic effects persisting at the end of 48 hours were taken for purposes of comparison, and the categories and methods of computing employed for the pyrethrins were used for evaluating the results. The results are given in Table III.

In Table III, in addition to the expression of the concentrations in weight of flower-heads, we have given the percentage of pyrethrin I present at each concentration. The data in this table are placed under three headings—A, B and C, for in addition to the samples cropped in 1927 and tested in 1928, there are figures given for the crop from two stations taken in 1926 and tested in 1927, and also a certain amount of preliminary data for the flowers grown at Swanley and Wye in 1927 and tested shortly after receipt in that year. Comparisons of the data should only be made within their appropriate groups as duplicate tests should be done within as short an interval as possible.

The toxic effects of the various samples in Group A can be graded in the following order: (1) Worcester—the most toxic sample; (2) Scilly Isles, Swanley, Wisley, Reading, Harpenden—not quite so toxic; and (3) Seale Hayne—least toxic. In the case of the Wye samples difficulties were encountered in placing the sprayed insects in their appropriate categories. We are readily able to separate the Worcester sample from the Seale Hayne and these two are the samples containing the largest and least amount, respectively, of pyrethrin. The intermediate samples cannot be separated from each other with any degree of precision, but in toxic values they definitely come between these two, as they also do in percentage of pyrethrin.

Group B of this table contains two samples whose toxicity data have been dealt with more fully elsewhere (1). It was there stated that it was highly probable that the differences found in the toxicity of the Scilly Isles and Wye samples of 1926 were significant. We have determined the content of pyrethrin in both samples and found that of the Scilly Isles sample to be materially higher than that of the Wye sample.

Group C contains data of a preliminary nature, the results of one set of experiments carried out in August 1927 on two samples harvested

Table III. *Toxicities to* A. rumicis *of pyrethrum flowers grown at different stations.*

(N = not affected, S = slightly affected, M = moribund, D = apparently dead.)

Marks awarded to each concentration = (½S % + ¾M % + D %).

Sample and origin	Concentration: In terms of part of plant gm./100 c.c.	Concentration: As pyrethrin I gm./100 c.c.	N %	S %	M %	D %	M and D %	Marks
	Group A*. Harvest, 1927. Tested, 1928.							
Harpenden (1)	0·5	0·0018	—	—	30	70	100	85
Pyrethrin I = 0·355 %	0·4	0·0014	—	—	40	60	100	80
„ II = 0·355 %	0·3	0·0011	—	—	43	57	100	78·5 (?)
	0·2	0·0007	25	20	45	10	55	37·5
	0·1	0·00036	85	10	5	—	5	2·5
	0·05	0·00018	100	—	—	—	0	0
Reading (2)	0·5	0·00195	—	—	20	80	100	90
Pyrethrin I = 0·39 %	0·4	0·0016	—	—	50	50	100	75
„ II = 0·33 %	0·3	0·0012	—	5	75	20	95	58·5
	0·2	0·00078	20	15	65	—	65	36
	0·1	0·0004	75	10	5	10	15	12·5
	0·05	0·0002	90	10	—	—	0	2·5
	0·025	0·0001	100	—	—	—	0	0
Scilly Isles (3)	0·5	0·0022	—	—	20	80	100	90
Pyrethrin I = 0·445 %	0·4	0·0018	—	—	40	60	100	80
„ II = 0·46 %	0·3	0·0013	—	5	55	40	95	69
	0·2	0·0009	25	5	55	15	70	44
	0·1	0·0004	60	25	10	5	15	16
	0·05	0·0002	80	20	—	—	0	5
Seale Hayne (4)	0·5	0·0014	—	—	70	30	100	65
Pyrethrin I = 0·28 %	0·4	0·0011	—	10	90	—	90	47·5
„ II = 0·31 %	0·3	0·00084	—	25	70	5	75	46
	0·2	0·00056	70	10	20	—	20	12·5
	0·1	0·00028	90	—	—	10	10	10
	0·05	0·00014	80	20	—	—	0	5
Swanley (5)	0·5	0·002	—	—	—	100	100	100
Pyrethrin I = 0·41 %	0·4	0·0016	—	—	40	60	100	80
„ II = 0·47 %	0·3	0·0012	10	—	55	35	90	62·5
	0·2	0·0008	35	10	55	—	55	30
	0·1	0·0004	100	—	—	—	0	0
Wisley (6)	0·5	0·0019	—	—	—	100	100	100
Pyrethrin I = 0·39 %	0·4	0·0016	—	—	40	60	100	80
„ II = 0·415 %	0·3	0·0012	5	5	85	5	90	49
	0·2	0·00078	45	10	30	15	45	32·5
	0·1	0·00039	55	10	35	—	35	20
	0·05	0·0002	100	—	—	—	0	0
Worcester (7)	0·5	0·0022	—	—	—	100	100	100
Pyrethrin I = 0·46 %	0·4	0·0018	—	—	27·5	72·5	100	87
„ II = 0·415 %	0·3	0·0014	—	2·5	25	72·5	97·5	85·5
	0·2	0·0009	10	—	55	35	90	62·5
	0·1	0·00045	50	15	35	—	35	21
	0·05	0·00022	90	10	—	—	0	2·5
Wye (8)	0·5	0·0018	—	—	50	50	100	75
Pyrethrin I = 0·375 %	0·4	0·0014	—	—	50	50	100	75
„ II = 0·325 %	0·3	0·0011	15	25	55	5	65	38·5 (?)
	0·2	0·0007	15	40	25	20	45	42·5 (?)
	0·1	0·00036	80	—	5	15	20	17·5
	0·05	0·00018	100	—	—	—	0	0
	Group B*. Harvest, 1926. Tested, 1927.							
Scilly Isles	0·5	0·0029	—	—	—	100	100	100
Pyrethrin I = 0·59 %	0·25	0·00145	—	—	—	100	100	100
„ II = 0·53 %	0·1	0·00058	—	—	40	60	100	80
	0·05	0·00029	—	10	75	15	90	55
	0·025	0·00014	—	40	60	—	60	40
Wye	0·5	0·00185	—	—	—	100	100	100
Pyrethrin I = 0·37 %	0·25	0·0009	—	10	5	85	90	90
„ II = 0·34 %	0·1	0·00037	20	5	30	45	75	61
	0·05	0·00018	65	15	10	10	20	19
	Group C*. Harvest, 1927. Tested, 1927.							
Swanley	0·5	0·00205	—	—	—	100	100	100
Pyrethrin I = 0·41 %	0·25	0·0010	—	—	—	100	100	100
„ II = 0·47 %	0·1	0·0004	—	—	30	70	100	85
	0·05	0·0002	—	20	30	50	80	70
Wye	0·5	0·0019	—	—	—	100	100	100
Pyrethrin I = 0·375 %	0·25	0·0009	—	—	—	100	100	100
„ II = 0·325 %	0·1	0·00037	—	30	10	60	70	72·5
	0·05	0·00019	50	20	—	30	30	35

* As insect resistance does not remain constant from year to year comparisons of results can only be made within each group.

a few weeks earlier; they are included because of the unsatisfactory data for the Wye sample when tested in 1928. The Swanley sample is slightly the more toxic, although the differences are scarcely significant, and it is noticeable that the content of both pyrethrins is higher in the Swanley than in the Wye sample.

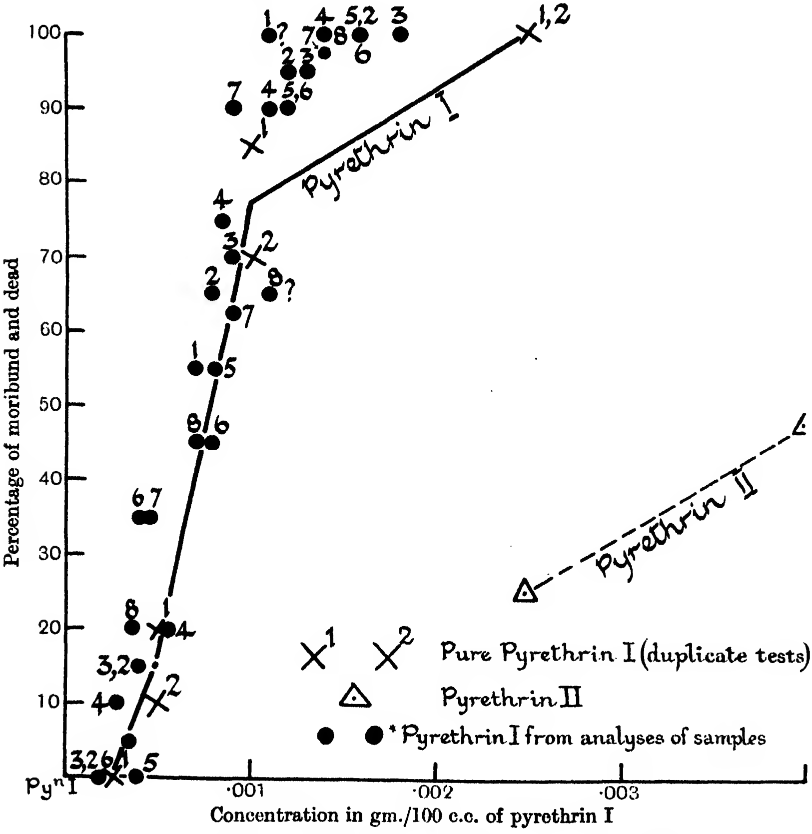


Diagram 2 *a*. Toxicity to *A. rumicis* of pyrethrin I in the isolated state, and as calculated from analysis of flower-heads. Toxicity evaluated in terms of percentage of moribund and dead insects. The numbers to the black circles correspond to numbers of samples in Table III.

Pyrethrin I was considered by Staudinger and Ruzicka to be mainly responsible for the insecticidal effect of pyrethrum; the data given above for the toxicity of the isolated poisons lead to the same conclusion. It appeared a practical method of testing the acid method of analysis, to plot the toxicity values for the above samples against the actual con-

centrations of pyrethrin I (calculated from the dilutions and the percentage amounts of pyrethrin I in the samples), upon the same diagram as the toxicity values of a specimen of the pure poison itself, and on the same scale. This would determine the degree of concordance. We have

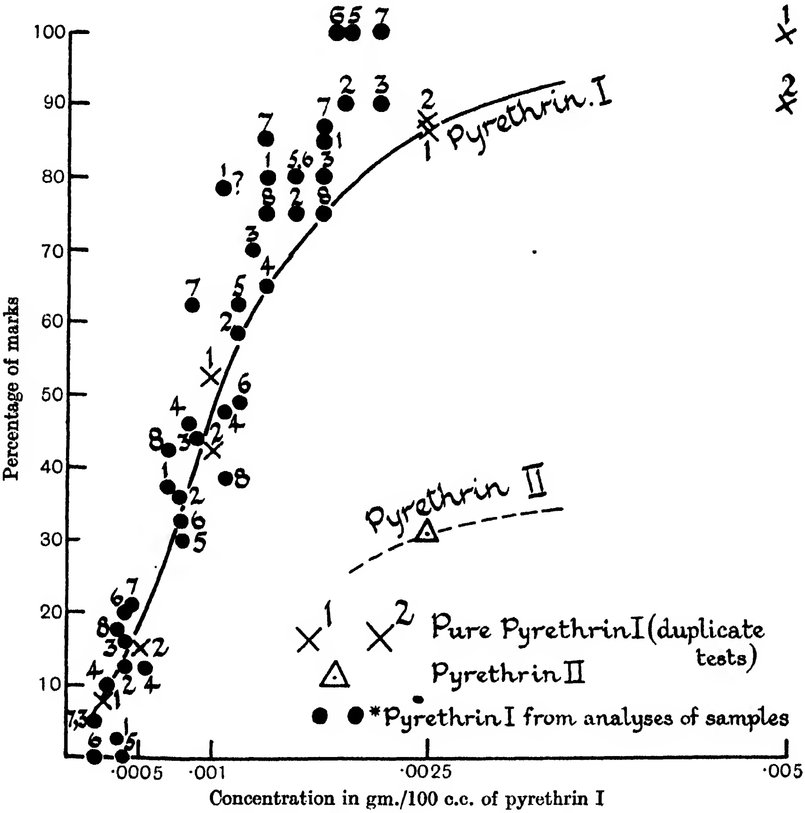


Diagram 2 *b*. Toxicity to *A. rumicis* of pyrethrin I in the isolated state, and as calculated from analysis of flower-heads. Toxicity evaluated by marks. The numbers to the black circles correspond to numbers of samples in Table III.

done this in Diagrams 2 *a* and 2 *b*, the numbers on the diagrams corresponding to the numbers given to the samples in Table III.

Owing to the rather arbitrary nature of evaluation, we have in these diagrams used both the methods of computing toxicity referred to on p. 270. In Diagram 2 *b* we have drawn the shortest smooth curve through the points for pyrethrin I which are marked by crosses. Taking

into account the minute concentrations which are effective, the points obtained for the various samples of pyrethrum fall significantly close to the curves indicating the toxicity of pyrethrin I. Towards the upper part of the curves there is an indication that the samples are more toxic than their content of pyrethrin I would suggest; it should, however, be noted that the curve for pyrethrin I itself may possibly ascend more steeply than shown owing to the rather wide separation of the points, but it is also probable that at these higher concentrations pyrethrin II plays a definite part in influencing the toxicity of the samples. On a diagram on this scale the toxicity of pyrethrin II is represented by only one point (shown by the triangle). We consider that the degree of concordance between the two sets of results is sufficient to demonstrate the value of the acid method, and particularly the determination of pyrethrin I, as a means of evaluating pyrethrum.

Staudinger and Ruzicka (*loc. cit.*) have indicated the possibility of a small amount of the acids being combined with other alcohols than pyrethrolone. If this were so, a lack of concordance between the analytical results and toxicity would be expected. The data presented, and the close agreement of the results given by the semicarbazone method, show that in our samples such compounds can only be present in traces, and their effect can probably be discounted. The possibility, however, suggests that from time to time the semicarbazone method or a direct estimation of toxicity should be used as confirmative tests.

The effect of external conditions upon the content of pyrethrin.

Pyrethrum being a perennial crop, it is a matter of importance to know how the toxic properties vary with the age of the plant. The data given in Table III gives a little preliminary information as to the variation in pyrethrum from year to year, *e.g.* the crop taken in Harpenden in 1927 has a percentage content of pyrethrin of 0·7 per cent., whereas in 1928 it has increased in plot 17 *c* to 1·0 per cent. and in plot 20 *c* to 1·28 per cent. The pyrethrin content of flowers taken in the Scillies in 1926 averages 1·1 per cent. but in 1927 the percentage had dropped slightly to 0·85 per cent. These variations are significant, and they cannot be related to differences in soil, or to the age of the plants as the effects for the two stations are in the reverse order. It may be tentatively suggested, that the meteorological conditions prevailing during a critical portion of the growth period have a bearing on the production of the toxic compounds in the plant. In Harpenden, at any rate, the

years 1927 and 1928 were climatically very different, 1927 being cold and wet with little sunshine, and 1928 dry and warm with considerable sunny periods.

With respect to soil conditions it is usually considered that poor calcareous soils are most suitable for pyrethrum. The plants we have tested were from a wide range of soils but our data are not sufficient to yield definite information as to the type of soil most suitable for this plant.

Conclusions.

It is evident from the data given, that, to certain insects, pyrethrin I is the most highly toxic contact poison at present known, and in our experience rotenone (tubatoxin) is the only compound that approaches it at all closely in toxicity. The results of our experiments agree too with those of Staudinger and Ruzicka in showing pyrethrin II to be less toxic than pyrethrin I and, although we find a relatively larger difference than they did, the discrepancy is almost certainly due to the wide differences both in the insects and technique employed. The important fact emerges that the contact insecticidal properties of pyrethrum are almost entirely due to the presence of pyrethrin I, and the aim of plant breeding should be to increase its amount.

Pyrethrum has the considerable advantage over most potent insecticides of being comparatively harmless to man, and there should be in consequence a large field of usefulness before it. Its use at present is circumscribed by its specificity of effect, certain insects being very resistant to it, and to the supposed readiness with which toxicity is lost. There is, however, no reason why its use should not be extended and this might be greatly facilitated if flowers of higher toxicity could be produced by systematic plant breeding.

For the latter purpose some suitable method of determining the percentage of poisons is necessary which should be reasonably rapid and simple. Hitherto most of the methods suggested have depended upon the determination of some value in no way correlated with, or a measure of, the amount of the toxic principle present. No method can be entirely satisfactory that does not definitely assess the amount of the poisons themselves. The micro-methods described above are adaptations of Staudinger and Harder's macro-methods, and care has been taken to test their correlation with toxicity determinations as accurately as possible. Bearing in mind the unique toxicity of pyrethrin I, the close accordance between the toxicities of the flower-heads, when calculated

to their content of pyrethrin I, and that of the isolated poison affords a striking confirmation of the validity of the results as given by the acid method. A further independent check is provided by the semicarbazone method, which depends on the estimation of the alcoholic constituent of the pyrethrins and has given results in substantial agreement with those obtained from a determination of the acidic constituents.

A suitable analytical method, in addition to its value for plant breeding for higher poison content, is likely to prove of use for standardising extracts. Again, the employment of the stalk of pyrethrum has up to the present been regarded as a questionable practice, as it contains a much smaller proportion of the toxic principles than the flowers; it nevertheless provides a potential source of the pyrethrins, if suitable methods of extraction could be devised. The data in Table II show that the percentage of petroleum ether extract of the stalk is much less than that of the flowers, and that in consequence, the petroleum ether extract of mixed stalk and flowers contains only a slightly lower percentage of pyrethrin than the extract of the flowers themselves. The weight of extract per unit weight of plant material being less in the case of the mixture, larger amounts of the mixed stalk and flowers than of the flowers would be required to give the same weight of dry extracts. The economic significance of this procedure would depend very largely on the increased cost of the transport, handling and extracting a larger bulk of material, but to some extent this would be offset by the saving of labour in detaching the flowers from the stalk, especially if a mechanical means of harvesting could be devised.

Whether the methods described can be used for detecting adulteration, can only be determined by further experience. The range of pyrethrin I content, even in samples of flowers grown from the same batch of selected seed, is variable and may possibly depend upon the meteorological conditions of the season, even when grown in the same bed. The percentage of pyrethrin is therefore not a constant for even genuine samples of the flowers. Combined with the methods elaborated by McDonnel, Roark, LaForge and Keenan (5) it may, however, prove itself of great value for purposes of detecting sophistication, for if the latter is suspected and a large sample is available, the examination of the acids and the determination of their constants should help in the detection of the most skilful adulteration.

It is important to realise, however, that after long exposure to damp conditions pyrethrum powder loses its toxicity and a genuine sample may be devoid of insecticidal value. Whether or not the chemical methods

outlined above will make it possible to detect such loss of toxicity, is a matter for further investigation.

We have used for this work samples of flowers grown in different localities but all raised from selected seed of the same origin[1]; considerable variation, therefore, was not to be expected. The biological tests were able to separate the samples with considerable precision into three classes of high, low and medium toxicity, but were unable to detect smaller differences. The chemical method was found to put the samples into the same categories and, in addition, was able to detect differences not distinguishable by the biological method.

Such small differences, as were found between many of the samples, are of little interest in practice, and it is important for the plant breeder to realise that samples grown from the same seed on the same soil can vary within fairly wide limits according to the season. The methods outlined should prove useful in any attempt to correlate toxicity with meteorological conditions, and in addition should be of value in studying the effect of soil conditions, manuring and cultivation upon the content of pyrethrin, and the useful duration of the plantation. These aspects of the problem of the extended production of pyrethrum call for further investigation.

The work here described forms parts of a co-operative investigation on English-grown pyrethrum as an insecticide (see 1), between the Plant Pathological Laboratory of the Ministry of Agriculture and the Rothamsted Experimental Station. We are indebted to the following for supplying additional material: South Eastern Agriculture College, Wye; Seale Hayne Agricultural College, Newton Abbot; The Horticultural College, Swanley; Research Station, East Malling; Research Station, Long Ashton; University of Reading; University College of South Wales, Aberystwyth; Royal Horticultural Society, Wisley; Experimental Station, Scilly Isles; Dept. of Agricultural Education, Worcester; Farm Institute, Sparsholt; Isle of Ely Demonstration Plot.

SUMMARY.

1. (*a*) Pyrethrin I and II have been isolated by the method of Staudinger and Ruzicka from the insecticidal plant Pyrethrum (*Chrysanthemum cinerariaefolium*). Both are shown to be highly toxic to the insect *Aphis rumicis*.

(*b*) Pyrethrin I was found to be the most toxic substance so far tested by us and, as it was about ten times as toxic to these insects as pyrethrin II, it is concluded that it is mainly responsible for the contact insecticidal value of pyrethrum.

[1] More recent work has indicated that the acid method is applicable to unselected seed from another source.

2. Two micro-analytical methods of determining the pyrethrin content are described. (*a*) By means of the acids after hydrolysis. (*b*) By means of the semicarbazone. They are given on pp. 278, 282.

3. The analytical results obtained for a series of pyrethrum samples agreed with their observed insecticidal properties to *Aphis rumicis*.

4. Comparisons of the pyrethrin contents, as estimated, with the results of direct toxicity experiments both on the pyrethrum samples and the pure pyrethrins, confirm the validity of the analytical methods.

5. There was a significant and positive correlation, in the samples tested, between the amounts of pyrethrin I and II.

6. Insufficient data are available to show a significant correlation between the size of flower-heads and the content of poison, or to draw conclusions as to the effect of external conditions such as soil, weather or age of bed.

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THE LEACHING OUT OF AUTUMNAL DRESSINGS OF NITROGENOUS FERTILISERS.

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(With Two Text-figures.)

The following investigation was prompted by the recent increase in the use of calcium cyanamide as a nitrogenous fertiliser, and the recommended use of it, in certain circumstances, as an autumnal dressing. It is regarded by most workers, and recent experiment and observation supports the view, as intermediate in action between nitrates on the one hand, and organic refuses on the other—that is in respect of rapidity of action and the time during which it can exert its effects.

It was decided to observe its behaviour by means of estimations of nitrate nitrogen in drainage or leachings from soil dressed with it, and from similar soil receiving other nitrogenous fertilisers in equivalent amounts and under the same conditions. Ammonium sulphate and rape dust were chosen as bases of comparison, the former as a common nitrogenous fertiliser which, while being to a certain extent fixed in the soil, is yet quick in action and the latter as a comparatively slow acting organic refuse. Observations were made throughout the winter 1926–7, and the experiment was repeated the following winter.

The experimental unit was a glazed earthenware pot with vertical sides 10 in. in diameter and 12½ in. in height, with a single tubulure at the bottom of the side. Each was fitted with a delivery tube and mounted on a stand so as to allow of a Winchester quart standing below it to receive drainage from the delivery tube. Eight such pots were prepared (12 in the second year) and a layer 1 in. deep of coarse washed gravel was put in each. An ordinary medium loam was partially dried indoors by spreading it on a clean floor, and, as soon as convenient, was put through a coarse sieve and thoroughly mixed; it was fed into the pots 10 lb. at a time with gentle pressing until each pot contained 40 lb. of soil. The pots were then set up with their receivers out in the open, so as to receive only rainfall by way of moisture.

A preliminary period was allowed for the soil to settle down and accumulated nitrates to be washed out by drainage before the fertilisers were added. The receivers were changed whenever 500 cc. or more of

drainage water had accumulated, and the nitrates in them were determined as soon as possible. During periods when water was left standing in the receivers a few drops of toluene was added to check the growth of algae.

Early in November the pots were grouped into four comparable groups (according to the amounts of nitrates washed out during the preliminary 2 or 3 weeks), and the fertilisers applied. Calcium cyanamide at the rate of 1½ cwt. per acre was taken as a basis: and equivalent doses of rape dust and ammonium sulphate were assessed from an accurate determination of their total nitrogen content.

Thus in 1926 the doses given were:

Rape dust (6·44 % nitrogen)	2·972 gm. per pot
Cyanamide (18·77 % nitrogen)	1·02 ,,
Ammonium sulphate (20·51 % nitrogen) ...	0·933 ,,

and in 1927:

Rape dust (6·58 % nitrogen)	3·15 gm. per pot
Cyanamide (17·92 % nitrogen)	1·15 ,,
Ammonium sulphate (20·72 % nitrogen) ...	1·0 ,,

The doses were mixed each with 10 gm. of fine sand before applying, and lightly scratched into the surface of the soil. In order to reduce the risk of direct washing-out of the fertilisers a margin 1–2 in. wide round the pot edge was left untreated.

On each occasion when the receivers were changed each one was well shaken, the volume of its contents was measured, and then allowed to settle clear once more. Occasionally it was necessary to filter before drawing an aliquot part for determination of nitrate content.

The nitrates were determined by the phenol disulphonic acid method. The experiment was continued throughout the winter (October–March) in each year, and terminated by the lack of drainage due to the greatly increased evaporation of March and April.

Towards the end of the second trial, on March 30th and April 25th, 1928, artificial watering was resorted to in order to obtain further figures.

The figures obtained from the duplicate pots in the first year, and the triplicate pots in the second year, agreed well in their general trend and ultimately were added together and, after multiplying those for the first year by $\frac{3}{2}$ to put them on a basis of comparison with those of the second year, were plotted to show the totals of nitrate nitrogen

leached out up to each date when the receivers were changed (Figs. 1 and 2).

The most striking point is that whereas in the winter of 1926–7 (Fig. 1) the greater part of the nitrogen remained in the soil until the

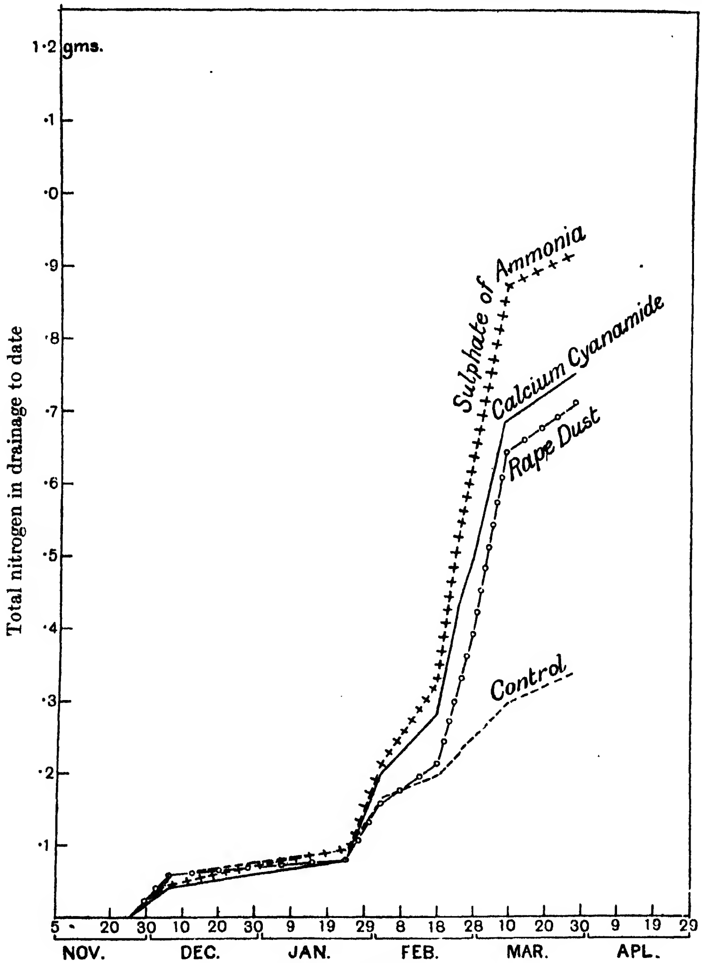

Fig. 1. 1926–7.

middle of February, quite two-thirds of the total had been washed out before that date in the subsequent winter (Fig. 2). The explanation is to be found in the rainfall curves; a period of low rainfall from the end of November (when the fertilisers were applied) to the end of January,

followed by a wet spell to the second week in March. In Fig. 2 the main position is reversed. High rainfall at the end of 1927 and in January 1928 washed out nearly all the nitrogen applied, and the lower rainfall

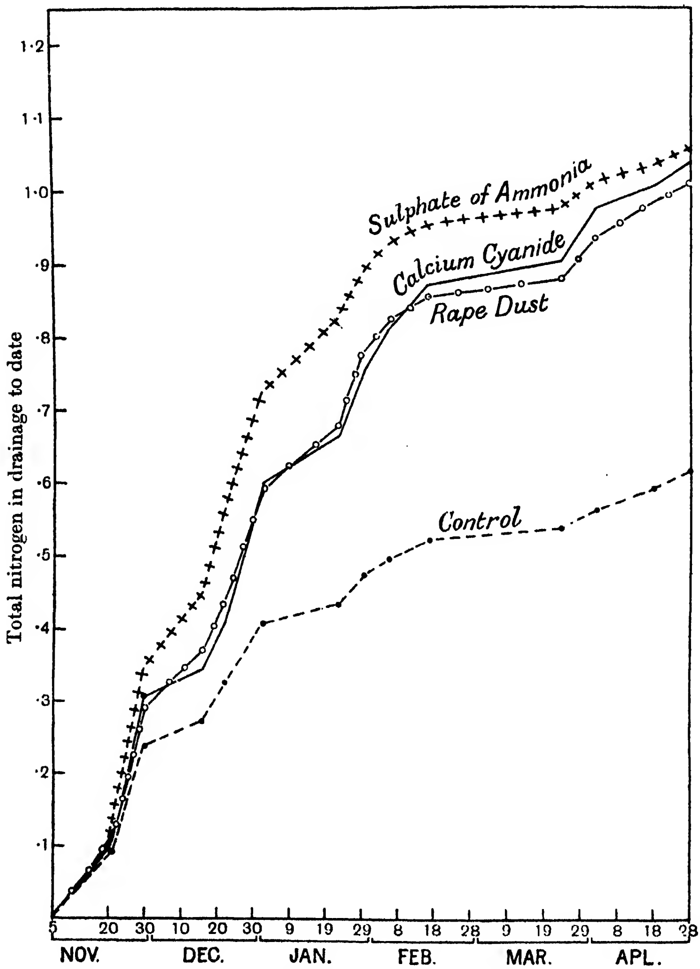

Fig. 2. 1927–8.

of the spring considerably reduced the drainage and lowered the individual nitrogen figures for that period.

It would appear further that under comparatively dry conditions there is not much to choose between these three fertilisers until late in January when the ammonium sulphate and cyanamide begin to leach

out rapidly, and the losses reach a maximum by about the middle of March. Under wet conditions, on the other hand, all three suffer losses rapidly from November onwards, though in this case the cyanamide and rape dust appear to be equally resistant.

SUMMARY.

The investigation bears out the conclusion of the Rothamsted experiments that leaching out may occur any time throughout the winter up to March, or rarely April.

The loss of nitrates is directly limited by the amount of drainage and so is indirectly determined by the rainfall, and by temperature—as it affects the rate of evaporation.

All three nitrogenous fertilisers are shown to lose well over 50 per cent. of a normal dressing by leaching out during the winter, but the loss is greatest in the case of sulphate of ammonia, least in the case of rape dust, calcium cyanamide being intermediate.

The writers wish to express their indebtedness to Miss N. Sugg who carried out the experimental work in the first winter.

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THE INFLUENCE OF FEEDING ON THE COMPOSITION OF MILK.
MANGELS VERSUS DRIED SUGAR BEET PULP.

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(With Two Text-figures.)

Amongst the numerous factors causing variation in the composition of milk, feeding is one which has received some considerable attention from past investigators. Many dairy farmers hold the opinion that a change of ration will exert an influence on the quality of milk, and there appears to be some foundation for this belief. Numerous "Feeding for Milk" experiments have been carried out at various centres in Great Britain and abroad, but the data obtained have given very conflicting results, due, it is suggested, to the operation of other factors than feeding, and also to high experimental error arising from the design and method of conducting the experiments.

In planning experiments of this type two important points should be observed, (*a*) the number of cows in each group must be sufficiently large to reduce to small proportions the error due to individuality, age, and period of lactation, and (*b*) the period of feeding each particular ration must be sufficiently long to minimise the effect of the fluctuation in composition of the milk, which is usually observed when cows are changed from one ration to another of a very different nature. There is a limit, however, to the duration of the experimental feeding period, since, in the case of lengthy periods, the inevitable change in climatic conditions will introduce a major factor into the experiment.

The method of interpretation of the data obtained calls for some comment. Usually the number of analytical determinations is limited, and even where daily records of composition are made from two groups of cows, the resulting data may not be of sufficient magnitude to allow for statistical treatment. In reviewing past "Feeding for Milk" experiments, one finds, in the majority of cases, that simple averages of the morning's and evening's milk composition figures have been taken, and conclusions based on these averages. In data of this type we are dealing

with two variables—yield of milk and percentage composition. From these two sets of determinations the weights of mild solids secreted at each milking should be calculated. By averaging the yield figures of milk and of milk solids, one can arrive at the true average composition of the milk obtained from each group of cows over the period of the experiment. Tocher[1] in his researches into variation in the composition of milk, has correlated yield and other factors with the weight of fat and solids not fat produced. Mackintosh[2], in a recent article, has pointed out that the percentage of fat calculated from the total weight of fat and of milk produced during a lactation, gives a more accurate result than that obtained by averaging the fat percentage figures from each milking. This method appears to have two advantages, (*a*) the two sets of figures —morning's and evening's milk—can be combined, and thus reduced to one set, and (*b*) the effect of yield of milk on the percentage figures of the milk constituents is eliminated. It is admitted that the average percentage composition figures resulting from this method do not represent the quality of the milk as it comes from the cow, but it is claimed that by this means a truer picture of the influence of a given factor, such as feeding, can be obtained, in that a differentiation between the effect on quality due to variation in yield alone, and that due to increased or decreased secretion of milk solids, is made. For the reasons stated above, this method of interpreting milk analysis data has been followed in the case of the experiments which form the basis of this paper.

With a view to testing the relative effect of mangels and dried sugar beet pulp in the winter ration of dairy cows, two experiments were laid down on Leicestershire farms in the early months of 1928, by Mr Thos. Hacking, the Agricultural Organiser for that county, to whom the design of the experiments is entirely due. Both herds of cows—non-pedigree shorthorns—were producing "Grade A" milk under highly efficient management.

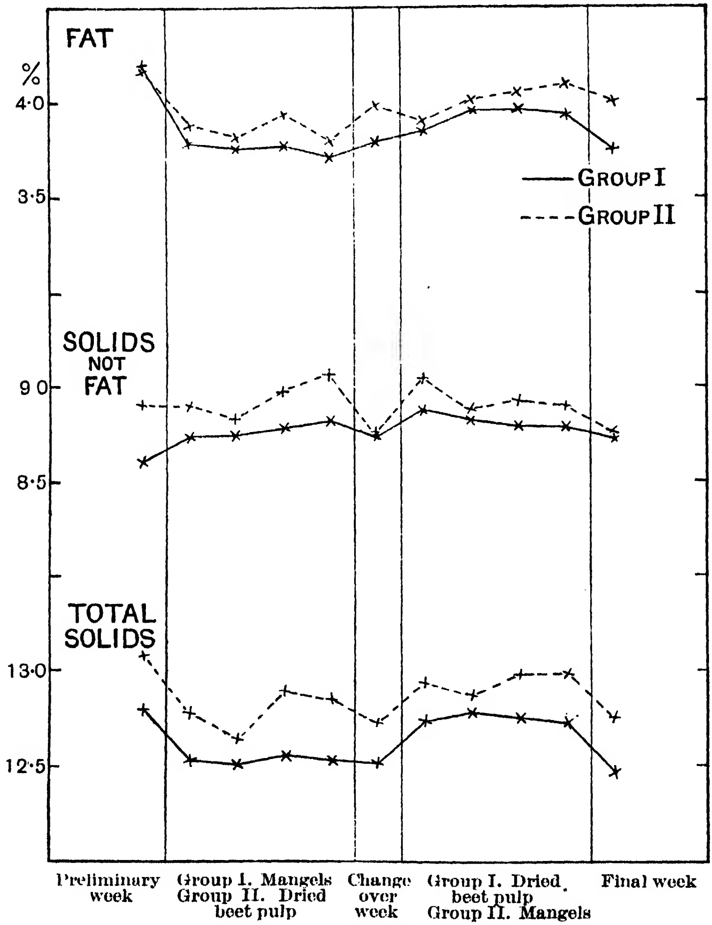
DESIGN OF THE EXPERIMENTS.

Centre 1. Two groups of ten cows each were reserved, the selection being made as evenly as possible, having due regard to yield, age, and period of lactation. By this one infers that, shortly before the experiment was due to commence, the available data concerning all the cows in milk in the herd were considered carefully, and two groups selected, which, on the average, gave approximately equal conditions relative to

[1] *Analyst*, **51**, No. 609, pp. 606–613.
[2] *Farmer and Stockbreeder*, June 25, 1928, p. 1405.

yield, age, and weeks in milk. The experimental feeding commenced on January 8 and the rations fed were as follows:

	Ration A		Ration B	
Per cow per day	Meadow hay ...	14 lb.	Meadow hay	14 lb.
	Mangels	32 „	Dried sugar beet pulp	4 „
3½ lb. per gallon	Dec: ground nut cake	2 „	Dec: ground nut cake ...	2 „
	Bean meal	2 „	Bean meal	2 „
	Maize germ meal ...	3 „	Maize germ meal ...	3 „
	Palm kernel cake ...	3 „	Palm kernel cake ...	3 „



Centre I. Weekly average percentage composition.

The rations were balanced so as to give approximately equal weights of starch equivalent and digestible protein to each group. The period of

feeding, and the particular ration fed to each group of cows, are given in the following table:

	Week ending	Ration for Group I	Ration for Group II
Preliminary week	Jan. 14	A	B
1st experimental feeding period	Jan. 21 Jan. 28 Feb. 4 Feb. 11	A	B
Change over week	Feb. 18	B	A
2nd experimental feeding period	Feb. 25 March 3 March 10 March 17	B	A
Final week	March 24	B	B

Centre 2. At this farm eight cows were selected for each group, the method of selection described previously under Centre 1, being followed. The experimental feeding commenced on February 12 and the following rations were used:

	Ration A		Ration B	
Per cow per day	Meadow hay ...	14 lb.	Meadow hay	14 lb.
	MANGELS	32 „	DRIED SUGAR BEET PULP	4 „
$3\frac{1}{2}$ lb. per gallon	Soya bean meal ...	2 „	Soya bean meal ...	2 „
	Maize germ meal ...	3 „	Maize germ meal ...	3 „
	Palm kernel cake ...	3 „	Palm kernel cake ...	3 „

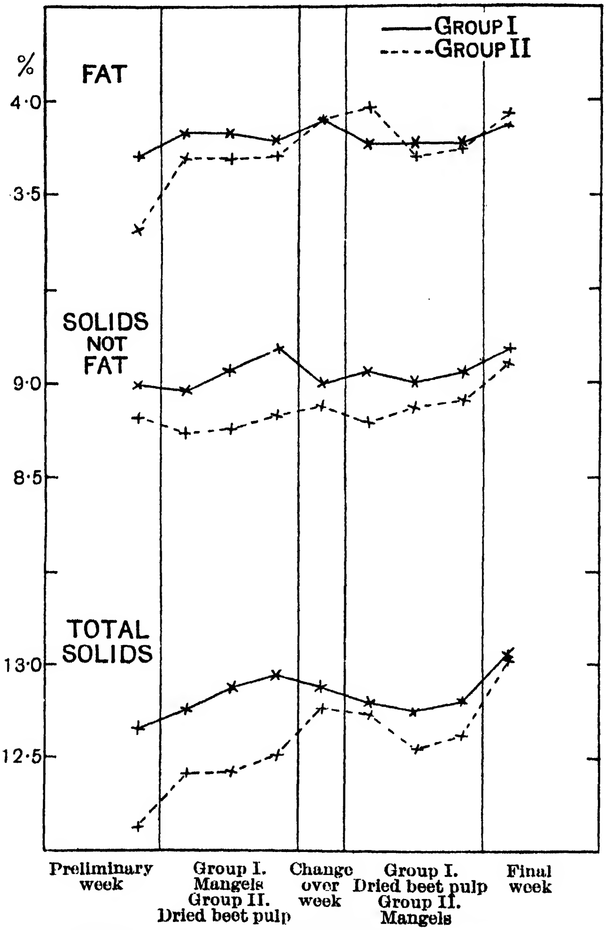
As at Centre 1, these rations were balanced as regards starch equivalent and digestible protein. Periods of feeding, and rations fed to each group, were as follows:

	Week ending	Ration for Group I	Ration for Group II
Preliminary week	Feb. 18	A	B
1st experimental feeding period	Feb. 25 March 3 March 10	A	B
Change over week	March 17	B	A
2nd experimental feeding period	March 24 March 31 April 7	B	A
Final week	April 14	A	A

The cows were out at grass during the final week.

The experiment at Centre 2 was not as successfully carried through as at Centre 1. In the "change over" week one cow in Group II fell sick and had to be removed. In consequence this group comprised seven cows only for the remainder of the period of experimental feeding. Owing to the warm spell of weather at the end of March, the cows became restive indoors, and it was found necessary to turn them out to grass before the commencement of the final week. These points will be referred to later in the discussion on the results. It may be emphasised

here that the author was not responsible for the design or carrying out of the experiments, and therefore is not in a position to discuss this section of the work. He is confident, however, that every care was taken to reduce, as far as was practicable, factors external to feeding, which might have influenced the yield and quality of the milk.



Centre II. Weekly average percentage composition.

Methods of sampling and analysis.

At six consecutive milkings each week—the milkings on Saturdays Sundays and Mondays being avoided—composite samples of the milk from each group of cows were taken. The milk from each cow was

weighed in a parallel-sided weighing bucket, and a sample of the milk taken by means of a metal tube. Determinations of fat by the Gerber method, and of total solids by evaporation, were made, subsequently, in the laboratory.

ANALYTICAL DATA.

Owing to limitation of space it is regretted that the full analytical data cannot be given. With the exception of a few abnormal results at the commencement of the experiment, the quality of the milk produced at Centre 1 was quite normal throughout the period of experimental feeding. No noticeable effect in changing over from Ration A to Ration B, and vice versa, was apparent.

At Centre 2 the quality fluctuated considerably during the first two weeks of the feeding, but became more steady later. The composition of the milk from this centre compared very favourably with that produced at Centre 1, being on the whole of good average quality.

It may be mentioned here that in all cases where very high or low results were recorded, duplicate analyses were made, and the results invariably confirmed.

YIELDS OF MILK AND MILK SOLIDS.

As previously observed, the weights of fat and solids not fat secreted at each milking were calculated from the yield of milk and percentage composition figures. A steady fall in the daily yields of these constituents is shown during the period of feeding, thus following the decline in the yield of milk. This positive correlation of yield of milk with yield of milk solids has been noted previously by Tocher[1]. At both centres a marked rise occurred in the yield of milk and of milk solids during the final week.

In considering the weekly average figures some interesting points evolve. The yield of fat fell steadily at both centres during the first feeding period, and to a certain extent during the second feeding period, thus following the decline in the yield of milk. In the "change over" week, however, a slight rise in the yield of fat is shown in three cases, the fourth giving the same average figure as in the preceding week. This variation from the normal fall of the preceding weeks indicates an increase in the secretion of fat during the "change over" week. It is suggested that this increased secretion was due to the stimulative effect of a sudden change of ration. Solids not fat secretion shows a more or less steady fall after the first week, but no check in the fall, as shown in the case of the fat data, is observed in the "change over" week figures.

[1] *Loc. cit.* pp. 608–609.

Percentage composition.

The average percentage composition of the milk for each week has been calculated from the average yield of milk and of milk solids. The graphs shown in Charts I and II have been drawn from these data. Any variations shown by these percentage composition figures indicate changes in the secretion of milk solids. The fat percentage graph for Centre 1 points to a steady increase in secretion of this constituent in the milk of both groups of cows, during the second feeding period, with the exception of the last week. In Centre 2, however, the corresponding graphs are erratic. Solids not fat percentages, in the case of Centre 1, give little indication of variation due to feeding, with the exception that a remarkable drop in composition occurred in the "change over" week for Group II. Group I graph shows a similar but smaller drop. The graphs drawn from the Centre 2 data tend to approach one another during the second period of feeding. In considering the total solids graphs, one observes that at Centre 1 the change from mangels to beet pulp (Group I) produced a much greater increase in secretion than in the case of Group II, where the change in feeding was in the reverse direction. This indication of an increased secretion of milk solids in favour of the beet pulp is small, but distinct. The total solids graph for Centre 2 does not show a comparable effect. As previously noted, the results obtained during the second feeding period of Group II at this centre must be accepted with caution, in consequence of the reduction of the number of cows in this group from eight to seven at the end of the "change over" week.

Summary of data.

Tables I and II sum up the data obtained from these experiments. By combining the results from the two groups of cows under each ration respectively, the error due to individuality, and the natural variation which always accompanies the advance in lactation, are considerably lessened. Perusal of these results indicates the following points:

1. Dried sugar beet pulp feeding produced a higher yield of milk and of milk solids than mangel feeding.

2. The average composition of the milk produced during the whole period of feeding each ration, respectively, is practically the same, the small differences shown being well within the limit of experimental error.

3. The secretion of milk solids was proportional to the yield of milk, with the exception that during the "change over" week a temporary stimulative effect was noticed.

Table I. *Total yields in lb. of milk, fat, solids not fat, and total solids obtained on three days per week during the two 4-week periods of feeding rations containing mangels and dried sugar beet pulp respectively to each of the two groups of cows, with the average composition of the milk produced by each of the two rations.*

CENTRE 1.

	Total yields in lb.				Average percentage composition		
	Milk	Fat	Solids not fat	Total solids	Fat	Solids not fat	Total solids
MANGEL RATION							
Group I	2471	92·92	216·59	309·51	3·76	8·77	12·53
Group II	2177·5	87·25	194·51	281·76	4·01	8·93	12·94
Total for the two groups	4648·5	180·17	411·10	591·27	3·876	8·884	12·760
DRIED SUGAR BEET PULP RATION							
Group I	2184·5	85·71	192·48	278·19	3·92	8·81	12·73
Group II	2697	104·20	241·86	346·06	3·86	8·97	12·83
Total for the two groups	4881·5	189·91	434·34	624·25	3·890	8·897	12·787

Table II. *Total yields in lb. of milk, fat, solids not fat, and total solids obtained on three days per week during the two 3-week periods of feeding rations containing mangels and dried sugar beet pulp respectively to each of the two groups of cows, with the average composition of the milk produced by each of the two rations.*

CENTRE 2.

	Total yields in lb.				Average percentage composition		
	Milk	Fat	Solids not fat	Total solids	Fat	Solids not fat	Total solids
MANGEL RATION							
Group I	1997	76·02	180·94	256·96	3·81	9·06	12·87
Group II*	1826·5	69·23	161·65	230·88	3·79	8·85	12·64
Total for the two groups	3823·5	145·25	342·59	487·84	3·799	8·960	12·759
DRIED SUGAR BEET PULP RATION							
Group I	1830	68·76	165·38	234·14	3·76	9·04	12·80
Group II	2138·25	79·05	187·25	266·30	3·69	8·76	12·45
Total for the two groups	3968·25	147·81	352·63	500·44	3·725	8·886	12·611

* Group II, mangel ration period, comprised seven cows only.

SUMMARY.

Experiments were carried out at two centres in Leicestershire during the spring of 1928, with the object of comparing the values of mangels and dried sugar beet pulp in the winter ration of dairy cows.

Two groups of cows were selected at each centre, and the usual

method of feeding for a period on one ration and then reversing, was adopted. Composite samples of the milk from each group of cows were taken at six consecutive milkings per week, during the period of the experiment. Determinations of fat and solids not fat were made, and the yield of milk at each milking recorded.

Although there are indications that the change of ration caused a slight temporary variation in the quality of the milk, the secretion of milk solids followed, in a general way, the variation in yield. This is confirmed by the average composition figures representing the whole of the milk from each particular ration.

Thanks are due to Mr Thos. Hacking for placing every facility for obtaining these data at the disposal of the author.

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A NOTE ON THE SAMPLING OF SUGAR BEET.

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INCONSISTENCY in the returns of sugar percentages from the factories has often perplexed growers and has at times given rise to some mistrust of the analytical procedure. Consignments of beet lifted on the same day and from the same field have been credited by the factory with widely different sugar contents. This has occurred within the writer's experience, even though the precaution was taken of loading the cart loads of beet into two trucks alternately, thereby eliminating any possibility of one truck being filled with beet from a better part of the field than the other.

An explanation is to be found in the small size of the samples actually analysed, the usual sample consisting of only ten beet. To represent accurately the bulk from which they are drawn, these ten roots must either contain the whole range of sugar percentages in the proportions in which they occur in the bulk, or else "fluke" such other combination as will give the same average sugar content. How seriously they may fail to do either is shown by experiments conducted for two years at the Norfolk Agricultural Station. The figures acquire additional interest by reason of the large number of experimental results which are published annually without any indication of their reliability.

The actual figures and the estimations of significance made from them cannot, of course, be applied to other occasions. They furnish, nevertheless, a useful example of the inadequacy of small samples of beet, at all events, for experimental purposes. It will come as a surprise to many that the analysed samples consisting of ten beet picked at random have not given results which could be relied on to within one per cent. of sugar either way. Since the whole of the twenty-five samples in each year were taken from an area only fifty-five yards square, it is not unreasonable to suppose that samples taken at bigger distances apart may be subject to greater differences of soil, and perhaps of other conditions also, and, consequently, liable to whatever variation such differences may cause.

The differences in sugar content between samples of beet may obviously be due either to hereditary causes or to effects of environment, or both. To the plant-breeder, searching for beet of higher sugar capacity,

the distinction is of importance. It also clearly demands consideration in the case of manurial or cultivation experiments. The ordinary grower, or even the Agricultural Organiser demonstrating large plots to farmers, is concerned only with the total variation which is liable to occur.

The variety of seed used in the experiments was Kuhn, sent out by the Cantley factory. Up to the present no opportunity has occurred of testing the uniformity in sugar content of the many varieties of sugar beet now available. Information on this point will doubtless be supplied in the report of the National Institute of Agricultural Botany on the variety trials now in progress.

Lay-out.

The experiments were laid out as five by five Latin Squares, each sub-plot being eleven yards each way, or exactly one-fortieth of an acre. The beet were drilled at eighteen inches between the rows, so that there were twenty-two rows on each sub-plot.

A sample was taken from each plot and analysed at the factory. Sampling was done by stretching a string diagonally across each plot and taking the plant immediately beneath or nearest to the string on alternate rows, omitting the two outside rows. Ten roots were thus lifted from each plot and formed one sample for purposes of analysis. They were conveyed immediately to the factory and analysed. The sugar percentages are shown in their respective squares in the tables. The letters C, W, X, Y and Z indicate different manurial treatments.

First Year.

	Sugar percentage, per plot					Total	Mean	
	C 17·7	W 18·0	X 17·0	Y 16·8	Z 17·3	86·8	17·36	
	Z 16·7	X 17·1	W 17·5	C 17·4	Y 17·5	86·2	17·24	
	W 16·7	C 17·3	Y 17·2	Z 17·3	X 17·8	86·3	17·26	
	Y 17·9	Z 16·9	C 17·0	X 16·6	W 16·7	85·1	17·02	
	X 16·0	Y 17·3	Z 17·5	W 16·8	C 18·0	85·6	17·12	
Total	85·0	86·6	86·2	84·9	87·3	**430·0**	86·00	
Mean	17·0	17·32	17·24	16·98	17·46	86·00	**17·20**	General mean

*Statistical analysis of results**.

Probable error of one result = 0·547.

„ mean of five results = 0·245.

„ difference of any two means = 0·346.

The significance of one result (2·18 × 0·547) = 1·194 % sugar.

„ mean of five results (2·18 × 0·245) = 0·534 % sugar.

„ difference of any two means (2·18 × 0·346) = 0·756 % sugar.

Second Year.

	Sugar percentage, per plot					Total	Mean	
	Y 18·1	W 18·4	C 18·2	X 18·8	Z 19·3	92·8	18·56	
	C 18·4	X 18·6	Z 17·4	Y 18·6	W 18·3	91·3	18·26	
	W 18·8	Z 19·0	Y 18·6	C 20·2	X 17·0	93·6	18·72	
	Z 19·0	C 20·1	X 18·3	W 19·3	Y 17·9	94·6	18·92	
	X 19·2	Y 18·9	W 18·8	Z 19·5	C 19·0	95·4	19·08	
Total	93·5	95·0	91·3	96·4	91·5	**467·7**	93·54	
Mean	18·70	19·00	18·26	19·28	18·30	93·54	**18·70**	General mean

Statistical analysis of results.

Probable error of one result = 0·608.

„ mean of five results = 0·272.

„ difference of any two means = 0·385.

The significance of one result (2·18 × 0·608) = 1·325 % sugar.

„ mean of five results (2·18 × 0·272) = 0·593 % sugar.

„ difference of any two means (2·18 × 0·385) = 0·839 % sugar.

Wide differences in sugar content were found between samples (of ten beet) taken within a few yards of each other. In the tables below the five samples from each manurial treatment are grouped together.

Sugar Percentages, by Manurial Treatments.

First year

	C	W	X	Y	Z
	18·0	18·0	17·8	17·9	17·5
	17·7	17·5	17·1	17·5	17·3
	17·4	16·8	17·0	17·3	17·3
	17·3	16·7	16·6	17·2	16·9
	17·0	16·7	16·0	16·8	16·7
Mean	17·48	17·14	16·90	17·34	17·14
Extreme difference	1·0	1·3	1·8	1·1	0·8

* For a description of the method of statistical analysis employed, the reader is referred to *Statistical Methods for Research Workers* (p. 234), by R. A. Fisher, Sc.D. (Oliver and Boyd).

Second year

	C	W	X	Y	Z
	19·0	18·3	17·0	17·9	19·3
	20·2	19·3	18·8	18·6	19·5
	18·2	18·8	18·3	18·6	17·4
	20·1	18·4	18·6	18·9	19·0
	18·4	18·8	19·2	18·1	19·0
Mean	19·2	18·7	18·4	18·4	18·8
Extreme difference	2·0	1·0	2·2	1·0	2·1

It will be seen that in only one case did all five samples fall within a range of 0·8 per cent. of sugar, while the greatest difference found between any two samples which had been similarly treated was 2·2 per cent. of sugar.

Since samples of ten beet have given such indefinite indications of sugar content, it is interesting to go further and see what improvements might be expected by grouping five such samples, and estimating the error of their mean. It will then be seen that the analysis of fifty beet, in groups of ten, has reduced the estimate of significance to 0·534 per cent. and 0·593 per cent. of sugar in the two experiments. This is still unsatisfactory but it is nevertheless a big advance on the smaller sample.

Conclusions.

The results of these experiments go to show that samples of ten beet cannot be trusted to give anything like a close indication of the true sugar percentage of a large number of beet. By increasing the sample to fifty a degree of precision was obtained which would probably be regarded as satisfactory for commercial purposes. As a measure of the effect of experimental treatments, however, even fifty beet have in this case proved quite inadequate. A general advisory policy based upon such data might be entirely misleading. It is, of course, possible that under other circumstances greater accuracy might be secured, and further information on the question of the size of samples is urgently required. Until such is forthcoming all experimental data should be submitted to rigorous statistical test before they are applied to general farm practice.

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THE DETERMINATION OF ORGANIC CARBON IN SOILS.

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In order to characterise a soil by laboratory examination, a knowledge of the amount of organic matter present is of great importance, for this constituent is not only the medium in which the micro-organisms of the soil fulfil their functions, but is also, together with the clay, the repository of the colloidal properties of the soil. Yet it must be admitted that, in ordinary routine, organic matter is either not determined or else expressed as loss on ignition, a quantity which at best is only an approximation and, in the case of soils containing much clay or calcium carbonate, may give a very misleading impression of the amount of organic matter actually present.

The reason for the neglect of this important determination is not difficult to understand. For a method of soil examination to approve itself for routine purposes, it must be fairly inexpensive on the score of apparatus readily operable by semi-skilled workers, and, in short, of such a character that it can be carried out in large numbers as required by soil survey or general advisory work. Most of the methods at present in use, or proposed for use, are based on the determination of organic carbon, with the use of a conventional factor for conversion to organic matter. The standard method for the determination of organic carbon is, of course, dry combustion in oxygen or air. This is an operation for skilled workers, requiring the use of a combustion furnace and the consumption of large quantities of gas. The wet methods, using chromic acid or potassium permanganate, appear to be only slightly less laborious, and give results which are not only smaller than those obtained by dry combustion, but are also not connected with these results by any fairly constant ratio. Further, in any method in which carbon dioxide from the oxidation of organic matter is determined, special measures have to be taken with soils containing carbonate, and the error where much carbonate and little organic matter is present may be considerable.

In the present paper we describe our attempts to utilise the oxidation of organic matter by sulphuric acid in the ordinary Kjeldahl digestion

as a basis for determining the organic carbon of soils. In this procedure, the principal reaction is the oxidation of organic matter to carbon dioxide and water. Since the hydrogen and oxygen are present approximately in the proportions in which they occur in water, the equation is essentially,

$$C + 2H_2SO_4 = CO_2 + 2H_2O + 2SO_2.$$

If we determine the amount of sulphur dioxide produced by this reaction, we obtain a measure of the carbon oxidised. We have attempted to do this by passing the gaseous products of reaction through standard iodine solution and titrating the excess iodine with standard sodium thiosulphate solution.

Since $C = 2SO_2 = 4I$, 1 c.c. then of N iodine $= 0{\cdot}003$ gm. carbon.

Experimental.

The sulphur dioxide method.

The apparatus consists of a long-necked Pyrex Kjeldahl flask, extreme length 30 cm., fitted with a rubber stopper, through which passes a short glass tube closed at the top with a piece of rubber tube and screw clip, and another tube which is connected with the absorption apparatus. This consists of a train of bulbs bent in the form of a V, of which the short limb is furnished with a large bulb of diameter about 7·5 cm., and the long limb with ten bulbs of diameter about 4·5 cm. The flask is connected to the end with the large bulb and the long limb with the smaller bulbs is clamped so that it lies at an angle of about 15° with the horizontal. We would not claim that this is the most convenient form of absorption apparatus. It may be that an absorbing tower of the Reiset type would be more convenient.

Fifty c.c. of semi-normal iodine solution are introduced into the absorption apparatus and diluted with about 100 c.c. of boiled distilled water. Into the flask is placed sufficient soil to furnish 0·02–0·05 gm. of carbon. In our earlier experiments, we found that, using soil ground to pass the 1 mm. sieve, appreciable amounts of carbonaceous material survived digestion. We therefore tried the effect of grinding our samples to pass a 100 mesh sieve and found that this difficulty disappeared. We have accordingly, in all the work described in this paper, used 100 mesh samples of air dry soil, and our results are expressed on the air dry basis. As our small 100 mesh samples are kept in stoppered bottles, the comparisons are not vitiated by changes in moisture content. Sulphuric acid (25 c.c.), ignited potassium sulphate (15 gm.) and copper

sulphate (0·3–0·4 gm.) are added and the flask is connected up with the absorption apparatus. The rubber tube on the short glass tube is securely closed with a screw clip. The contents of the flask are cautiously heated. When all danger of frothing is over, the flame is turned up sufficiently to maintain the contents of the flask in vigorous ebullition. In practice, this means an ordinary bench bunsen at about three-quarter heat. If the heating is too vigorous, there is danger of charring the indiarubber stopper. With care, there is no charring, even after many determinations. It is, of course, necessary to use perfectly clean stoppers. New stoppers should be freed from adhering powder. Digestion is continued for one hour after the contents of the flask have assumed their final colour. The gas flame is then turned out and the contents of the flask are allowed to cool for about half a minute in order to obtain a slight negative pressure in the flask. The screw clip is now opened and a long glass tube of such a diameter that it is securely clasped by the rubber tube but passes through the short glass tube is introduced until its lower end is just above the surface of the contents of the flask. The apparatus is now connected up to an aspirator and aeration is carried out for 20 minutes, during which time about 15 litres of air are drawn through. We may mention that in our earlier experiments we had a glass tube reaching down into the flask throughout the digestion, but we found that the contents of the flask got into it, so that some of the soil escaped oxidation.

At the conclusion of aeration, the contents of the absorption train are washed into a tall 600 c.c. beaker, and the excess iodine is titrated with semi-normal sodium thiosulphate. The end-point is sufficiently sharp without the use of starch paste. From time to time a blank experiment is performed, and the amount, generally from 0·6 to 0·9 c.c. of semi-normal iodine, is subtracted from the amount of iodine reduced as shown by the thiosulphate titration. All determinations are in duplicate. Agreement is generally within 2 per cent. The following results obtained on a single soil indicate the amount of variation to be expected:

Pasture soil: 5·49, 5·53, 5·52, 5·61, 5·45, 5·46 per cent. carbon.

Dry combustion method.

The results obtained by the above method are compared with the results obtained on the same soils by dry combustion. A few remarks are necessary in description of our method of carrying out

this operation. We use an ordinary hard glass combustion tube, 950 mm. × 12 mm., packed as follows. At the front is a copper gauze coil, 120–150 mm. long. Behind this is about 300 mm. of mixed granular and wire-form cupric oxide retained in position by asbestos fibre plugs. Then follows the soil in a porcelain boat, about 60 mm. long. After this comes a cupric oxide coil, 120–150 mm. in length. The method of heating is as in ordinary organic combustion, the boat being heated last. The products of reaction are drawn through standard baryta (about 0·2 *N*) in a Reiset absorption tower and the excess baryta titrated with 0·2 *N* hydrochloric acid. A second tower is connected up as a check, but we rarely find any appreciable amount of carbon dioxide in it after combustion. Combustion lasts about 1½ hours, during which about 5 litres of dry CO_2-free air are drawn through the apparatus. In the case of soils containing carbonate, the combustion is continued for a sufficient period to ensure that all the carbon dioxide from the decomposition of carbonates is evolved. About 5 hours suffices. The completeness of the decomposition is tested by carrying on the heating for a further period with fresh baryta. A blank experiment is performed from time to time and the amount of carbon found subtracted from the results actually obtained. The blank varies from 0·0010 to 0·0013 gm. carbon. With sucrose, replicate determinations have given 41·8, 42·3, 41·9, 41·4 and 41·9 per cent. carbon as against 42·1 per cent. carbon required by theory. All results are the mean of duplicates, which generally agree within 1 per cent., except in the case of peats where, presumably owing to errors of sampling, the difference is rather greater.

Comparison of results by the two methods.

Comparing the results by the two methods as shown in Table I, it will be seen that, although the figures expected from the combustion data were not obtained by the sulphur dioxide method, there is yet a tendency for the percentage recovery to approximate to a steady value. For the British soils, mainly Welsh, but including a wide variety of types, the average recovery is 89·6 ± 1·03 per cent. For the four foreign soils, exactly the same average recovery is indicated. We therefore suggest provisionally that the organic carbon may be obtained by multiplying the result obtained by the sulphur dioxide method by 100/89·6 = 1·116. It is, of course, realised that the soils examined are only a selection, and that much wider divergences might be obtained in an extended series of comparisons.

Table I. *Percentage carbon by dry combustion and by sulphur dioxide method for different soils.*

Soil	Percentage combustion	Carbon SO_2 method	Recovery by SO_2 method	Remarks
Madryn X	3·80	3·38	88·9	Light loam, Caernarvon
Y 113	1·985	1·77	89·2	Sandy loam, Yorks.
A 122	3·85	3·48	90·4	Medium loam, Anglesey
Ab. M. 1	3·875	3·375	87·1	Heavy loam, Caernarvon
Bodrwyn	5·175	4·625	89·4	Medium loam, Anglesey
Lledwigan	4·31	3·81	88·4	Light loam, Anglesey
G 167	3·36	3·105	92·4	Sandy loam, Glamorgan
M 9	3·22	2·96	91·9	Medium loam, Monmouth
Greensand	1·375	1·23	89·5	Sandy loam, Surrey
Keuper	1·28	1·155	90·2	Clay loam, Warwicks.
G 165	3·68	3·27	88·9	Light loam, Glamorgan, $CaCO_3$ 0·70 %
D 105	2·07	1·80	87·0	Medium loam, Denbigh, $CaCO_3$ 8·0 %
G 109	4·405	4·00	90·8	Clay loam, Glamorgan, $CaCO_3$ 4·7 %
Ab. M. 2	3·56	3·13	88·0	Heavy loam, Caernarvon
G.L.	6·35	5·51	91·5	Gravelly loam, Caernarvon
Peat moss	44·05	38·6	87·6	Peat moss litter. Ignition 82·6 %
Gwernygof	37·7	33·95	90·1	Mountain peat. Ignition 52·0 %
Cwmdu	21·6	19·85	91·9	Heath peat. Ignition 40·25 %
Average for British soils: 89·6 ± 1·03 %.				
Tshornosem	3·055	2·775	90·8	Black earth, Ukraine
Alberta	3·525	3·115	88·4	Black prairie soil
Tarouba	1·475	1·32	89·5	Clay alluvium, Trinidad
Rendzina	2·84	2·55	89·8	Czechoslovakia, $CaCO_3$ 5·7 %
Average for foreign soils: 89·6.				

Results with pure substances.

It may be of interest to give some figures obtained with pure substances. These are shown in Table II.

Table II. *Percentage of carbon in pure substances by sulphur dioxide method compared with theory.*

Substance	Percentage theory	Carbon % by SO_2 method	Percentage recovery
Sucrose	42·1	39·6	95·5
Starch	44·4	42·4	95·5
Cellulose	44·4	41·9	94·4
Carbon	100·0	93·3	93·3
Humic acid	45·5	39·8	85·3*

* Artificially prepared from peat. Carbon determined by combustion.

It will be noticed that, with pure substances, the recovery is higher than in the case of soils. The humic acid on the other hand, gives a lower recovery. It may be that the actual recovery with soils represents an intermediate value between the higher figure for fresh organic matter and the lower figure for humified material. The degree of humification may be of significance in this connexion.

Advantages of sulphur dioxide method.

We venture to direct attention to some advantages of the proposed method over the dry combustion and wet combustion methods at present in use. In the first place, the manipulation and apparatus are fairly simple. Apart from the absorption train, which can be made for a few shillings, all the apparatus is such as would be found in any soil laboratory.

Secondly, this procedure has the advantage over methods which involve the collection of carbon dioxide that it is at once applicable to carbonate soils without the necessity for correcting for carbonate carbon. Knowing the irregular distribution of carbonates in soils, the determination of small amounts of organic carbon in the presence of much carbonate would seem to be subject to considerable error.

Thirdly, the method can be conveniently combined with the determination of nitrogen by the Kjeldahl method. The contents of the digestion flask can be distilled with strong soda in the usual way. The amount of nitrogen determined is, of course, less than in ordinary Kjeldahl procedure, but we have found no difficulty in obtaining closely agreeing replicate results, using $N/50$ acid with methyl red as indicator. Incidentally, with this method, the Kjeldahl digestion can be performed without the necessity for using a fume chamber. The carbon and nitrogen can be conveniently determined on the same sample of soil in one series of operations. It is proposed to use this method in a study of the carbon-nitrogen ratio of a number of soils differing in fertility and agricultural history.

Determination of carbon dioxide produced by Kjeldahl digestion.

It seemed of interest to us to examine to what extent the carbon dioxide evolved during the sulphuric acid oxidation corresponded with the amount of sulphur dioxide found. We therefore in some preliminary experiments connected up a baryta tower to the iodine bulbs. Although good agreement was found between the amount of carbon dioxide indicated by baryta absorption and the sulphur dioxide found by iodine, we decided, for convenience in co-operative work, to determine the carbon dioxide by separate procedure. This consisted in connecting up the digestion flask with a tower containing 25 per cent. sodium bichromate solution, followed by a tower containing standard baryta. We ascertained that the bichromate removed the sulphur dioxide completely. The carbon dioxide absorbed by the baryta was determined by titration in the usual way. It should be added that a longer period of aeration,

namely 45 minutes, was necessary to bring over all the carbon dioxide evolved. The aspirated air was, of course, freed from carbon dioxide. In Table III we give the percentage recovery by this method compared with the corresponding figures by the sulphur dioxide method.

Table III. *Comparison of recovery by sulphur dioxide and carbon dioxide.*

	Percentage recovery by SO_2	Percentage recovery by CO_2
Tshernosem	90·5	86·9
Madryn X	88·9	88·1
Y 113	89·2	90·7
A 122	90·4	92·5
Ab. M. 1	87·1	88·4
Bodrwyn	89·4	88·8
Alberta	88·4	90·7
Tarouba	89·5	95·9
G 167	92·4	87·9
M 9	91·9	92·8
Average	89·8	90·3

Although the individual results do not show complete concordance, the average recovery by the two methods is strikingly close. The carbon dioxide figure is of course put up by the abnormally high figure for the Tarouba (Trinidad) soil. We may reasonably conclude that the sulphur dioxide produced corresponds with the amount of carbon oxidised. A further reason for examining this point was that we apprehended that soils containing inorganic reducing substances would give more sulphur dioxide than would correspond with the oxidation of organic carbon, rendering the proposed method inapplicable. This would be the case with certain marsh soils containing sulphides. For example, a lacustrine peat gave the following results:

Combustion	SO_2 method	Recovery	CO_2 method	Recovery
27·64	27·60	99·9 %	24·2	87·5 %

Doubtless more extreme instances might have been found. For such soils the carbon dioxide-baryta variant of our method might be recommended. If carbonates are also present there is, of course, an additional difficulty.

The factor for converting organic carbon to organic matter.

The object in determining the organic carbon of a soil is to estimate its content of organic matter. For this purpose the factor 1·724 is commonly used. We do not propose to enter into an extended discussion of this question, and hesitate to make any concrete proposals for a

change. We give, however, in Table IV, some results for four peats in which the loss on ignition may be taken as giving a fairly close idea of the amount of organic matter.

Table IV. *Percentage of carbon in organic matter of certain peats.*

	Ignition loss	Combustion carbon	Percentage carbon in organic matter
Peat moss	82·6	44·0	53·3
Corsybol	52·0	27·65	53·2
Gwernygof	70·0	37·7	53·9
Cwmdu	40·25	21·6	51·4

The close agreement of the first three is worthy of notice. The lower figure for the Cwmdu peat may be due to the figure for loss on ignition exaggerating the amount of organic matter actually present. In any case, it seems likely that the conversion factor should be greater than 1·724 and this point requires further investigation. If we take the mean of our figures, we obtain 1·888 as conversion factor. Combining this with the factor recommended for obtaining organic carbon by the sulphur dioxide method we obtain 2·107 as the factor for converting these results to organic matter. A fair approximation would be obtained for most purposes by simply multiplying by 2.

General Discussion.

We may infer, from the correspondence between the carbon dioxide and the sulphur dioxide produced in the Kjeldahl digestion, that the deficit from complete recovery is due to some of the organic matter being converted into incompletely oxidised and undecomposable compounds by the sulphuric acid-potassium bisulphate digestion. We have tried the effect of increasing the time of digestion but found no appreciable increase in recovery. There is also a tendency to bumping on long continued digestion. In some of our earlier experiments, we found that the use of ferric oxide accelerated the disappearance of the organic colour during digestion. Further investigations, however, showed that it had no effect on the final result and we have therefore not felt inclined to suggest any change from the ordinary Kjeldahl procedure. We propose to investigate further the possibility of increasing the recovery by modification of the details of the digestion.

It will be remembered that the percentage recovery varies somewhat in different soils. This, of course, is only to be expected since the organic matter in soils cannot in all cases be in the same state of oxidation. It may be that when a large number of results are accumulated, it will be

possible to distinguish somewhat, and to use a different factor for different types of soils. For the present it would seem that the use of the factor recommended, 1·116, will not involve any serious error as a first approximation.

We recognise that the proposed method only gives an approximate figure for the organic carbon of soils, but we feel justified in putting it forward for consideration, because a method which can give results to within 2–3 per cent. of the truth represents a considerable advance on the present position so far as routine work is concerned. Experience with standardising methods for mechanical analysis suggests that before a method can be recommended for general use it must be tried on a large number of soils from all parts of the world. We have tried to use as representative a selection of soils as possible; but it is too much to hope that there are not some soils for which our method may prove unsuitable. For example, soils such as marsh soils containing sulphides would certainly be liable to give high results by the sulphur dioxide method. Before admitting the method into routine it would be advisable to have a large number of comparisons on widely differing soils. For ordinary well-oxidised soils, however, the method as described may be used with a reasonable degree of confidence.

NOTE.

Since completing the work described in the foregoing paper, we have found a tower absorber to be much more convenient. It consists of a tube 50 cm. long, 2 cm. internal diameter, fitted with three platinum gauze grids in the lower half to break up bubbles. The tube is fitted into a 250 c.c. filter flask. The titration can be carried out in the flask, about 50 c.c. of water sufficing for washing out the tower. We find that about 3 litres of air suffices for aeration and that the blank correction is almost negligible.

SUMMARY.

(1) It is proposed to estimate the amount of organic carbon in soils by determining the amount of sulphur dioxide produced in the ordinary Kjeldahl digestion. The gaseous products of reaction are passed through standard iodine solution, and the excess iodine titrated with standard sodium thiosulphate. Details of the method are given.

(2) The results obtained with a number of soils of differing character and origin are compared with the figures obtained for organic carbon by dry combustion. The sulphur dioxide method gives results which

average 89·6 ± 1·03 per cent. of the combustion figures. It is proposed therefore that the percentage of organic carbon found by this method should be corrected by the factor 100/89·6 = 1·116.

(3) The percentage recovery of carbon indicated by the proposed method is rather higher for pure substances but still falls short of 100 per cent.

(4) The proposed method is applicable to carbonate soils without the necessity for any correction for inorganic carbon.

(5) It is likely that soils containing inorganic reducing substances such as sulphides will give high results by the proposed method.

(6) Absorbing the sulphur dioxide in 25 per cent. sodium bichromate, it is possible to determine the carbon dioxide by passing the gases through standard baryta in a Reiset tower. The organic carbon thus indicated agrees with that by the sulphur dioxide method.

(7) From data with certain peats, it appears that the factor 1·724 for converting organic carbon to organic matter is too low.

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"SINGLE VALUE" SOIL PROPERTIES: A STUDY OF THE SIGNIFICANCE OF CERTAIN SOIL CONSTANTS. II. STUDIES ON NATAL SOILS.

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1. Introduction.

In the first paper of this series (10)[1], Keen and the present writer discussed work by earlier authors on the approximate specification of the nature of a soil by a single soil constant, in place of a detailed analysis, and described experimental work designed to show the significance of a number of simple physical measurements. The chief objects of the present paper are to describe the results obtained with such measurements as applied to a number of Natal soils, and to discuss the value of some other easily obtained physical data as a means of specifying the nature of a soil. Work of this type was discussed at the International Congress of Soil Science at Washington in 1927, and it was resolved that co-operative work on an international basis should be undertaken.

It is unnecessary to repeat here the survey of the earlier work, which was given fairly fully in Paper I, but it will be convenient to refer briefly to a few of the most recent publications on the subject.

While it is desirable to use factors of the widest possible utility, it is evident that the choice of soil constants must be influenced by the purpose for which the data are required. Thus, Terzaghi (13), considering the soil as the foundation for engineering works, suggests that the compressibility, permeability, and consistency of the soil should be taken as the factors of main importance. Such measurements are doubtless closely correlated with the "colloid status" of the soil, and could be estimated by the methods developed by Atterberg (3) and by Haines (6). They would also be of great importance in studies of hard-pan formation, but they would probably not be so generally applicable to the specification of a normal soil as are some of the properties discussed earlier. It is interesting to note that Terzaghi remarks that the interpretation of the mechanical analysis of a soil is rendered difficult by the influence of the irregular shapes of the particles. This question of soil heterogeneity is one of considerable importance, and the custom

[1] Subsequently referred to as Paper I.

of expressing the results of a mechanical analysis in terms of "Stokesian" equivalent radii has until recently been apt to obscure the drastic nature of the assumptions made. Apart from this difficulty, arising from the irregularity of shape of the soil particles, the wide range of particle size leads to complications in the interpretation of such factors as the pore space of a soil, and it is, indeed, surprising that the actual field results show even approximate agreement with calculations carried out by various workers on the "ideal" homogeneous soil.

Harland and Smith (7) have found that the apparent specific gravity of a soil measured *in situ* by the Israelson (8) method gives useful information with regard to soil type. They find, for example, that the apparent specific gravity is greatest for sandy, light coloured, and mature soils. Results obtained in this paper for pore space—which is, of course, closely correlated with apparent specific gravity—seem to confirm this conclusion.

Davis and Adams (4), and other workers at the United States Bureau of Soils, have continued the work on the measurement of moisture equivalent, percolation, colloid content (by various methods), pore space, penetration, and plastic range. All these quantities specify useful soil characteristics, and are valuable soil constants. As stated in Paper I, however, the moisture equivalent is not available in many laboratories, on account of the expensive apparatus required; and, further, Veihmeyer and Oserkovsky (14) have pointed out that the results are seriously influenced by the precise technique adopted. It appears, therefore, that the moisture equivalent must be excluded from the list of simple physical properties available for approximate and rapid classification of a soil.

2. Soils used.

In the present investigation, 66 soils of varied type were used. Most of them were collected within a radius of 50 miles of Pietermaritzburg, particularly in the wattle-growing areas, but a few were obtained from more distant parts of Natal, or from Zululand. The material, therefore, does not represent such a wide geographical distribution as that examined in Paper I, but it was thought desirable to test the broad results obtained earlier by applying them to the case of a group of soils derived from one particular neighbourhood. At the same time, the soils examined show wide variations in their constitution; the clay content ranges from about 5 to 50 per cent., and other properties show a corresponding variation. It should however be stated that the samples were taken

mainly from areas of similar climatic conditions at altitudes ranging from 2500 to 4500 ft., the majority being between 3500 and 4000 ft.

Table I.

Locality	Description
Table Mountain Sandstone:	
1. N.T.E. Exp. Stn. Seven Oaks	Grey-brown sandy loam (virgin soil)
2. ,,	,,
3. ,,	,,
4. Broadmoor Estate, Wartburg	Brown sandy loam
5. Greytown District, West	Grey sandy loam
6. ,,	,,
7. Krantzkop	,,
8. Dalton	Grey sandy soil
9. ,,	Grey yellow sandy soil (2nd ft. of 8)
10. ,,	Yellow sandy soil (3rd ft. of 8)
11. Melmoth District, Zululand	Grey sandy loam
12. ,,	Grey brown sandy loam (2nd ft. of 11)
13*. ,,	Brown loam
14. See 1	Grey-brown sandy loam
15. Melmoth District, Zululand	Grey sandy loam
16. Greytown District, East	,,
17. ,,	Grey-brown sandy loam (2nd ft. of 16)
18. ,,	Yellow brown to red, sandy clay loam (3rd ft. of 16)
19. ,,	Brown sandy loam
20. Ahrens	Grey coarse sandy loam
21. ,,	Grey sandy loam
22. ,,	Grey brown sandy loam (2nd ft. of 21)
23. ,,	Yellow sandy clay loam (3rd ft. of 21)
24. ,,	Grey sandy loam
25. Greytown District, East	Light brown loam
Table Mountain Sandstone and Ecca Shales:	
26. Seven Oaks	Dark grey brown sandy loam to clay loam
27. Ahrens	Brown clay loam
28. See 1	Grey-brown sandy loam
29. ,,	Brown sandy loam
30. ,, (limed plot)	,,
31. ,,	Brown-yellow sandy clay loam (2nd ft. of 30)
32. ,,	Yellow sandy clay loam (3rd ft. of 30)
33. ,, (unlimed control to 30)	Brown sandy loam
34. Seven Oaks	Dark grey-brown loam to clay loam
35. ,,	Dark brown clay loam
36. ,,	Brown to yellow clay loam (2nd ft. of 35)
37. ,,	Yellow red clay loam (3rd ft. of 35)
Ecca Shales:	
38. See 1	Grey brown clay loam
39. ,,	,,
40. Greytown District, N. East	Dark grey brown clay loam
41. Seven Oaks	Dark brown clay loam
42. Richmond	Brown clay loam
43. Greytown District, N. East	Dark grey clay loam
44. Krantzkop†	Yellow brown clay loam
Ecca Shales and Dwyka:	
45. Richmond	Light grey clay loam

* Of mixed origin, Ecca Shales and Dolerite also present in this region.
† In Table Mountain Sandstone region.

Table I (*contd*).

Locality	Description
Ecca Shales and Dolerite:	
46. N.U.C. Grounds, Pietermaritzburg	Grey sandy loam
47. Scottsville, Pietermaritzburg	,, (virgin)
48. Seven Oaks*	Grey sandy clay loam
49. Richmond	Dark brown loam
50. ,,	Chocolate clay loam (virgin)
51. Seven Oaks	Dark brown clay loam
52. New Hanover	Brown clay loam
53. Seven Oaks	Chocolate brown loam
54. ,,	Dark brown clay loam (2nd ft. of 53)
55. ,,	Red yellow clay loam (3rd ft. of 53)
56. ,,	Chocolate brown clay loam
57. Greytown District, N. East	,,
58. ,,	Brown clay loam
59. Seven Oaks	Dark brown clay loam
Dolerite:	
60. Richmond	Chocolate loam
61. ,,	Yellow brown loam
62. Greytown, N. East	Chocolate loam
63. ,,	Red clay loam (2nd ft. of 62)
64. ,,	Dark red clay loam (3rd ft. of 62)
65. Seven Oaks	Chocolate brown clay loam
Granite:	
66. Piet Retief (Transvaal)	Yellow grey sandy loam

* In Table Mountain Sandstone region.

The soils belong in the main to three geological types—Table Mountain Sandstone, Ecca Shales, and Dolerites. Like most South African soils, they are highly deficient in phosphates, and phosphatic manuring wherever tried has produced very great increases in yield. None of them contains any considerable amount of lime. All the soils were passed through a 1 mm. (I.M.M.) sieve before use. The main features of the soils, and their sources, are summarised in Table I.

3. Experimental.

The determinations made were as follows:

Mechanical analysis (for purpose of obtaining clay content) (C).

Moisture content of air-dry soil (A).

Moisture content in equilibrium with an atmosphere of 50 per cent. relative humidity (R).

Loss on ignition (I).

Moisture content at sticky point (S).

Keen-Raczkowski box data:

(1) Moisture content of saturated soil (w).

(2) Pore space (p).

(3) Swelling (v).

In addition, the Haines shrinkage experiment was carried out with half the soils, and from this were calculated:

(1) Pore space (P).

(2) Apparent specific gravity (σ).

(3) Total shrinkage (T).

The experimental procedure was similar to that described in Paper I, but additional data are provided by the mechanical analysis and the Keen-Raczkowski experiment (9). Results are referred to the air-dry soil basis.

The replication of sticky point determinations was very satisfactory: the difference between duplicate determinations by one worker was seldom greater than 2 per cent., and the difference between results obtained by two independent workers was only slightly greater, as shown in Table II, which may be taken as typical:

Table II.

Difference in S between workers A and B	0–1	1–2	2–3	3–4	4–5	5–6	6–7	>7
No. of soils	8	8	5	1	2	1	3	0

As the experimental work was nearly complete before the publication of the revised (1928) A.E.A. method for mechanical analysis (2), the soil fractions are given as defined by the 1926 A.E.A. procedure (1): *i.e.* ignited fractions expressed as percentages of the air-dry soil. In Robinson's notation (12), for Coarse Sand, $Lv > 8 \cdot 87$; for Fine Sand, $Lv > 7 \cdot 70$; for Silt, $Lv > 6 \cdot 0$; for Fine Silt I, $Lv > 5 \cdot 0$; for Fine Silt II, $Lv > 4 \cdot 0$; for Clay, $Lv > 3 \cdot 0$. (This method of fractionation has the incidental advantage in the present work of making the clay fractions directly comparable with those given in Paper I.) An end-over-end shaker was not available, and this part of the dispersion process was carried out by bubbling air through the suspension for 24 hours (or more); the bubbling was sufficiently vigorous to keep all but the coarsest material in constant agitation. In the sampling process, the special tap pipette was used. Since the fraction is obtained by washing out the pipette with distilled water after discharging the soil suspension as completely as possible, and adding the washings to the discharged liquid, the following method was adopted in calibrating the pipette: The pipette was filled with a strong solution of sodium chloride of known concentration, and this volume of solution determined by weighing the salt obtained after evaporation of the solution and washings from the pipette. This method gives a value for the capacity higher than that

Table III.

					"Box" data			Mechanical analysis						
Soil	*A*	*R*	*I*	*S*	*w*	*p*	*v*	Coarse sand	Fine sand	Silt	Fine silt I	Fine silt II	Clay	Loss on solution
1	2·56	1·89	9·8	37·8	35·8	44·3	9·1	46·4	11·5	4·4	—	5·0	17·6	0·9
2	2·01	1·93	11·7	40·9	35·7	44·8	9·2	45·8	12·4	4·8	2·2	3·3	14·8	1·4
3	2·11	1·96	10·5	36·7	36·3	44·8	9·6	47·4	10·0	4·8	2·4	1·2	17·4	1·0
4	1·76	2·09	10·4	36·5	35·0	46·1	9·6	35·8	13·1	6·0	—	4·7	23·2	1·5
5	3·28	2·66	10·9	35·4	39·7	47·2	10·3	37·5	13·6	6·2	3·4	2·2	20·5	0·9
6	2·63	2·77	13·1	40·9	41·7	48·9	10·7	31·9	18·6	5·8	2·7	2·1	19·6	1·6
7	1·58	1·63	8·3	36·5	33·7	43·7	8·9	40·0	20·1	5·2	2·9	2·1	16·8	0·7
8	1·01	1·27	3·9	20·4	22·7	34·8	6·0	57·6	17·3	5·3	2·8	2·9	5·7	0·6
9	0·86	0·72	3·0	21·6	19·9	33·7	4·2	59·2	17·8	4·8	0·9	3·6	7·4	0·3
10	0·85	0·73	2·9	19·5	20·9	33·8	5·0	59·1	15·7	4·6	0·4	2·9	10·3	0·2
11	1·49	1·89	9·7	37·3	34·7	44·2	10·1	51·3	14·6	4·6	2·6	0·9	12·7	1·0
12	2·38	1·56	6·6	31·4	33·5	44·8	7·6	56·7	13·8	4·6	1·2	1·6	11·5	0·9
13	3·83	3·89	17·4	52·8	46·7	52·2	10·2	16·9	12·7	6·3	2·1	4·3	31·1	2·3
14	2·28	2·09	9·1	40·5	36·6	45·0	8·4	46·7	10·7	6·0	3·1	2·1	17·0	1·0
15	2·18	2·29	10·1	42·8	38·6	48·3	9·7	45·1	18·3	3·2	1·9	1·9	13·6	1·0
16	3·41	3·08	11·2	45·3	47·0	53·0	12·5	38·2	16·5	6·0	3·1	—	17·2	2·0
17	3·29	2·69	9·3	38·4	42·4	50·7	6·5	39·1	17·6	5·5	2·3	4·1	16·3	1·4
18	2·08	1·95	7·8	29·8	31·9	44·4	3·4	35·3	19·3	6·5	0·9	2·9	21·3	0·9
19	2·18	1·53	6·6	35·3	35·5	47·6	9·7	52·6	12·9	5·0	3·1	—	16·3	0·9
20	2·39	2·32	11·0	38·0	40·4	46·3	12·1	46·5	11·2	6·7	1·5	3·1	13·8	1·1
21	3·02	2·16	9·7	45·3	38·7	47·2	8·1	37·1	15·1	5·3	3·1	2·4	16·7	0·9
22	1·95	1·35	6·3	28·2	33·2	43·6	6·3	48·7	16·1	8·1	1·7	2·7	16·2	0·6
23	1·97	1·35	5·5	28·3	33·4	46·1	6·4	37·1	20·4	9·1	3·4	4·9	17·7	1·4
24	2·51	2·21	10·9	48·4	40·4	46·4	11·3	50·6	11·6	3·9	1·7	1·9	13·6	0·7
25	1·37	1·24	7·2	30·8	27·8	39·9	4·8	43·6	21·2	7·2	—	2·7	13·8	0·8
26	3·92	3·35	16·5	47·8	45·7	51·2	10·9	12·9	18·3	7·0	3·1	2·4	32·5	1·2
27	2·83	3·34	16·5	50·7	47·8	52·8	12·1	18·1	15·6	7·3	2·2	1·9	30·4	1·7
28	3·74	2·32	9·1	42·6	40·6	47·7	8·6	19·1	24·6	7·2	2·6	3·4	25·1	1·6
29	2·45	1·89	7·9	39·8	37·8	46·5	7·0	12·0	42·9	8·3	5·8	4·1	14·1	0·6
30	3·37	2·49	11·9	38·9	39·9	48·4	7·6	30·1	12·1	5·9	2·7	2·2	23·6	1·5
31	3·48	2·28	11·0	37·4	38·5	47·5	7·5	32·7	12·4	7·9	2·2	3·1	25·8	1·3
32	2·61	1·93	9·0	33·5	36·9	46·2	6·2	29·2	14·6	6·0	5·2	3·6	25·2	0·6
33	3·76	2·45	13·2	39·8	40·7	50·0	7·8	29·2	12·4	6·7	3·1	2·4	25·8	0·8
34	4·24	3·33	16·5	43·7	44·9	51·7	11·3	13·3	19·6	8·6	4·0	2·7	28·2	1·6
35	3·45	3·21	16·3	48·4	45·0	51·1	10·7	10·9	14·0	8·1	4·6	0·9	36·4	1·9
36	3·83	2·91	13·5	41·3	42·2	50·3	7·5	12·2	15·6	7·1	3·4	2·5	37·2	1·1
37	4·17	2·71	11·3	42·7	50·6	56·5	8·3	10·9	15·4	12·5	4·0	5·0	36·6	0·6
38	4·58	3·91	18·2	54·7	51·0	52·1	13·4	9·1	11·2	7·4	2·2	15·5	25·8	0·9
39	4·44	3·48	17·5	53·2	52·9	53·1	12·7	11·4	12·7	8·3	2·3	2·9	35·4	1·1
40	5·70	4·50	15·7	54·7	56·4	58·0	16·8	8·2	9·9	13·2	6·6	4·1	35·8	1·7
41	5·00	4·04	20·4	56·9	51·8	54·6	11·7	4·8	7·9	8·4	3·2	4·0	42·8	1·9
42	4·28	4·81	18·4	50·3	52·1	54·8	9·5	12·0	13·0	8·4	5·2	2·2	30·6	3·3
43	7·63	4·86	17·8	62·7	56·3	57·9	16·4	3·5	10·7	13·2	4·3	2·6	35·9	2·3
44	4·10	3·25	16·2	49·3	48·4	51·8	11·8	11·1	15·8	9·1	2·8	3·4	35·4	1·5
45	2·69	3·49	14·2	42·0	40·1	49·4	10·6	9·8	16·6	10·9	6·4	1·6	35·2	1·4
46	3·79	3·73	6·8	29·7	42·5	49·6	10·8	5·4	12·8	19·5	9·9	7·2	26·2	1·0
47	2·24	2·05	6·4	31·7	39·7	49·2	6·1	8·3	14·5	22·2	12·9	8·8	18·9	0·7
48	4·80	3·19	14·1	46·6	48·2	52·9	15·0	14·6	17·1	7·9	0·6	5·8	31·5	1·7
49	6·51	6·56	24·9	74·2	74·2	64·7	15·9	3·2	10·2	10·4	8·8	5·3	27·2	2·5
50	5·44	4·84	19·4	65·5	65·3	56·0	13·4	1·0	6·6	11·0	8·1	5·5	38·3	1·1
51	3·96	0·73	23·2	56·0	49·5	50·9	15·6	3·0	6·5	9·8	3·5	14·6	34·5	1·4
52	7·47	5·49	22·2	67·8	70·5	58·3	18·5	2·6	4·5	7·6	4·3	4·8	40·4	2·7
53	7·40	5·27	18·1	65·3	61·6	58·5	15·6	3·3	8·6	11·1	5·3	3·1	38·0	2·3
54	5·34	4·65	16·3	53·7	51·7	56·7	10·3	4·0	9·2	11·9	3·6	3·9	41·3	1·1
55	5·17	4·22	13·2	47·3	50·3	58·4	7·8	4·9	10·2	12·3	4·2	4·1	42·1	1·0
56	6·40	6·53	23·3	62·7	61·0	58·1	17·3	2·8	6·1	9·8	5·3	4·1	37·0	2·2
57	9·07	4·29	17·5	65·0	55·8	56·0	14·1	1·6	6·6	4·7	3·1	4·0	48·0	0·3
58	9·07	4·84	20·1	65·4	55·7	58·0	12·8	5·9	10·1	6·2	3·2	6·2	34·2	1·4
59	4·88	4·06	18·1	49·9	48·2	52·7	13·1	7·7	12·0	12·1	4·8	2·9	34·2	1·9
60	5·56	4·48	21·0	56·0	67·0	61·9	12·6	5·7	8·1	18·4	8·8	8·8	26·3	2·0
61	5·49	4·58	25·3	60·1	60·7	58·2	13·9	5·5	9·8	8·8	6·4	6·2	32·3	1·6
62	4·62	3·39	17·2	53·9	45·8	53·7	11·1	15·7	16·2	5·5	1·7	6·4	30·2	0·7
63	4·48	2·77	11·4	42·7	40·6	52·9	6·9	14·8	16·6	6·5	2·3	2·7	33·9	0·7
64	5·99	2·46	10·3	40·7	39·5	52·7	7·0	13·6	17·8	7·5	4·3	4·0	33·0	1·0
65	6·75	5·39	20·2	60·9	59·4	58·2	14·0	3·0	7·7	11·0	5·0	3·9	37·5	2·5
66	1·11	1·57	6·6	26·3	28·2	39·1	4·6	43·7	19·5	5·1	1·7	1·3	17·5	0·3

given by the ordinary method, but it is the one required in the present case. On account of the construction of the pipette, it is unlikely that its effective capacity will be the same as the nominal value, and the difference may be great enough to affect the final results unless the true capacity is found by calibration[1]. The sampling depths for the standard temperature of 15° C. were corrected for the prevailing laboratory temperature in each case. As the temperature was frequently as high as 24° C., the correction may be as great as 25 per cent.

The Keen-Raczkowski perforated box experiment was carried out in the ordinary routine manner; the boxes were cylindrical, 1 inch diameter, and 1 inch deep. As suggested in Keen and Raczkowski's original paper, the soil crumbs were broken up by passing the 1 mm. sieved soil through a finer (100 mesh) sieve, and then re-mixing the two fractions.

4. Discussion of Experimental Results.

The chief data are presented in Table III: from these, various correlation coefficients are calculated, and certain conclusions drawn. It will be convenient to consider first the confirmatory evidence derived from the present work for the conclusions reached in Paper I, and to deal subsequently with additional effects noted.

(*a*) *Confirmatory evidence in support of earlier results.* An examination of the figures in the table shows that, in nearly all cases, there is little difference between the values of A and R; of course, R is the more exactly specified quantity, and there is no need to discuss further the significance of A, beyond stating that it will give less accurately the information obtainable from R.[2]

In Paper I it was shown that there were definitely significant correlations connecting C, R, I and S. These results are fully confirmed by the present data, as will be seen from the set of correlations given in Table IV.

Table IV.

	I	R	S
C	0·751	0·733	0·743
I	—	0·828	0·928
R	—	—	0·859

Remembering that each of these correlation coefficients is calculated from 66 pairs of values, so that a correlation of 0·3 has a probability of more than 100 to 1 (5), there can be no doubt that the figures represent

[1] For example, two nominal 20 c.c. pipettes used in the present work gave effective capacities of 20·9 c.c. and 20·4 c.c.

[2] Compare Paper I for a fuller discussion.

real connections between the factors concerned. The coefficients are, in fact, higher than those obtained in the corresponding cases in Paper I, where only 39 pairs of values were examined. In both cases the IS coefficient is the highest in the table; and, in both cases, also, the CR coefficient is high.

A rather surprising feature in the present case, however, is that the RI and CS coefficients are much higher than would have been expected from the earlier set of data. The explanation of this effect is to be found in a consideration of the different types of soils used in the two sets of experiments. The Natal soils are decidedly heavier than those previously examined (Average Clay, $\bar{C} = 26{\cdot}1$ per cent., as compared with 19·8 per cent.), and they also show very much greater losses on ignition (Average Loss on Ignition, $\bar{I} = 13{\cdot}2$ per cent., as compared with 7·2 per cent.). It is almost certain that these high losses on ignition are not due wholly to organic matter, but in part—and possibly in large part—to "unfree" water, which is not removed by ordinary drying in a water oven, or over concentrated sulphuric acid. That this is the case is shown, for example, in the case of soil No. 49, which, despite its very high loss on ignition (24·9 per cent.), apparently responds to nitrogenous manuring. Thus, the values of I probably represent considerable amounts of water in addition to true organic matter, and this water is, of course, closely correlated with the water measured by the factors R and S. The presence of these apparently anomalously high correlations is thus explained: the explanation is indeed of the same type as that advanced in Paper I to explain the appearance of certain significant correlations on peroxide-treated soils, where no correlation was present in the original soils. There is therefore no real discrepancy between the two sets of data.

Unfortunately, no data are at present available for estimating the unfree water included in the value of I. One possible procedure is suggested by the results of the earlier work on soils treated with hydrogen peroxide. If the measurement of I were repeated after treating the soils with hydrogen peroxide, and the assumption made that 75 per cent. of the organic matter is removed by such treatment[1], then the residual effect of the water could be calculated.

It was further shown in Paper I that the sticky point moisture is correlated more closely with I than with C, and this result also received satisfactory confirmation from the present data: partial correlations are as follows:

$$r_{IS\,.\,C} = 0{\cdot}837, \qquad r_{CS\,.\,I} = 0{\cdot}189.$$

[1] This point is fully discussed in Paper I.

Another result was that R is mainly controlled by the clay content; the high correlation given in Table IV ($r_{CR} = 0{\cdot}733$) is in accordance with this result: but the confirmation is less convincing when partial correlations are taken. It is found that $r_{CR \, . \, I} = 0{\cdot}306$. This correlation is still definitely significant, and only slightly lower in probability level than the figure (0·672) obtained with the 39 samples, but a higher value might be expected. This figure is the only discrepancy of importance between the two sets of data.

The correlations between I and S and between C and R respectively are sufficiently close to justify an attempt to develop equations connecting the two pairs of variables. It is found that I and S can be connected by the equation[1]

$$S = 2{\cdot}05\, I + 17{\cdot}6 \pm 3{\cdot}1.$$

The equation connecting C and R is

$$C = 9{\cdot}1\, R \pm 5{\cdot}8.$$

In this second case, however, three soils—Nos. 49, 51 and 56 (all belonging to the Ecca Shale and Dolerite group)—give results for C so widely divergent from the calculated values that it appears most reasonable to ascribe the errors to some special cause, and to discard these three results in calculating the probable error. If this is done, the equation for the remaining 63 soils fits considerably better:

$$C = 9{\cdot}1\, R \pm 4{\cdot}3.$$

(*b*) *Mechanical analyses, geological formation and topography.* In Table III, the soils have been grouped for convenience of reference, in accordance with the underlying geological formations. An examination of the figures will show at once that the type of soil produced is very closely related to the underlying parent material. Thus, almost all the soils derived from Table Mountain Sandstone contain from 50 to 60 per cent. of "sand" (*i.e.* fine sand plus coarse sand), and less than 20 per cent. of clay. They are described from field observations as sandy loams: the colour of the surface soil is grey, brownish-grey, or, occasionally, reddish, and the subsoils vary from yellowish sandy loam to yellow-brown or reddish sandy clay.

The Ecca Shales tend to produce heavy soils; they contain from 15 to 25 per cent. of sand, and the average clay content is about

[1] This equation is in general agreement with the conclusion in Paper I, that about 16 per cent. of the water in S is accounted for by the non-colloidal soil constituents: for taking I as a measure of the colloid status of the soil, $S = 17{\cdot}6 \pm 3{\cdot}1$ when $I = 0$.

35 per cent. These soils are usually brown loams, on yellowish brown clay loam subsoils.

Between these two groups of soils are those derived from mixed Table Mountain Sandstone and Ecca Shale, and these contain 30 to 40 per cent. of sand, and about 30 per cent. of clay.

The characteristic of a Doleritic soil is the evenness of texture in the first three or four feet, and the red colour which appears in the second foot. The clay content (about 30 per cent.) is rather lower than in the case of the Ecca Shales soils, but the silt content is higher, and the sand content is about the same. These soils are chocolate-brown on the surface, with reddish subsoils. The mechanical analysis of soil No. 60 is typical of this group of soils.

There are further a number of soils which belong to the general Ecca Shales formation, but contain also intrusions of igneous Dolerite; they are, on the whole, intermediate in properties between the Ecca Shales and the Dolerite soils proper, although some of the clay contents are exceptionally high.

The Table Mountain Sandstone (Silurian) and Ecca Shales (Permian) series are both of great geological age, and most of the soils exhibit fairly well developed profiles, so that they might be regarded as more or less mature. They would probably be grouped in the Russian system of classification as "podsols." The closeness of the association between the soil properties and the geological formation is, from this point of view, rather surprising. The explanation, however, is to be found in an examination of the topographical features of the country, which is hilly and broken, and the rainfall conditions. In most of the localities from which the soils were obtained, a considerable proportion of the annual precipitation (which totals about 40 inches) is accounted for by the mists during the summer months[1], but there are in addition frequent heavy falls of rain (or, occasionally, of hail) of short duration, amounting to from 1 to 2 inches in an hour. Precipitations of the latter type lead to serious erosion of the soils, and, if such conditions have persisted through long geological ages, it is obvious that the soils will be less mature than would be supposed from a consideration of their geological history alone. Further, the rainfall is insufficient to produce great leaching: on the hillsides, most of the rain falling during a heavy shower runs off, causing erosion, but penetrating only a very short distance into the ground. Work is now in progress, from which it is hoped that it will be possible to estimate the significance of the profile formations

[1] It is well known that the best wattle growing areas are in the "mist belt."

under the topographical conditions prevalent in Natal and other parts of South Africa outside the central plateau.

Another very important effect of rainfall conditions should be noted. Almost the whole of the annual precipitation occurs during the summer months and, except under very unusual circumstances, the soil is never saturated. The result is that the ill effects of large quantities of clay seldom arise; on the contrary, there is a great advantage gained on account of the water retained by the clay (R in the preceding discussion). A soil which in a climate such as that of Eastern England, for example, would for the greater part of the year be water-logged and appear so heavy as to be practically unworkable, can under the actual conditions be ploughed and cultivated to give a satisfactory seed bed ensuring good growth of the young plant.

(*c*) *Water retained in the Keen-Raczkowski box.* It is evident from an inspection of the figures in Table III that the water retained in Keen and Raczkowski's perforated box is very closely related to the sticky point. This conclusion is stressed by the very high correlation between the two quantities: $r_{Sw} = 0{\cdot}935$. Further, the two quantities are not only proportional to one another, but very nearly equal: the arithmetic means for the 66 soils are $\bar{w} = 44{\cdot}2$ per cent. and $\bar{S} = 44{\cdot}7$ per cent. It appears, therefore, that w and S provide two different methods for measuring the *same* soil constant, and the conclusions that have been drawn with respect to the physical significance of S can be applied also to w. We shall see later that the perforated box experiment provides certain other significant data for the specification of a soil, and there are good reasons for suggesting that this experiment might usefully be adopted as the first stage in the systematic examination of a soil for which no data, other than field observations, are available. The main objections to this procedure would be the rather tedious work involved in sieving the soils and "packing" the boxes, and the fairly large amounts of soil required (about 50 gm. for each determination), and the fact that the experiment requires at least two days for its completion—one day for the saturation of the soil, and one day for the subsequent oven drying. None of these objections arises in the measurement of the loss on ignition, or the sticky point (using samples of about 10 gm. in the latter measurement, 8 hours' oven drying is sufficient). On the other hand, these two latter quantities do not provide such full information as the complete box experiment. The following alternatives are therefore suggested:

(1) If it is required to obtain rough information, from a single soil

factor, concerning the nature of a soil, or to determine whether there are significant differences in properties between a number of different samples, this can be done most easily and satisfactorily by a determination of the sticky point or of the loss on ignition.

(2) As the first stage in the systematic examination of a soil, the perforated box experiment should be performed.

In this latter case, it will not, as a rule, be necessary to carry out the sticky point determination as well, except as a check on other results.

(*d*) *The measurement of pore space.* In the introductory paragraph of this paper, reference was made to the difficulties arising in the estimation of the pore space of a soil, and in Paper I an example was given of the discrepancies between the interpretations of the results obtained by different methods. It was then pointed out that, while the pore space (p) had been shown by Marchand (11) to bear a close relationship to the clay content of the soil, no similar relationship was obtained when the clay content was compared with the pore space (P) of a well-kneaded block as determined by Haines' method (6). The explanation suggested for the discrepancy was that in the case of the measurements by the latter method, the particles were much more closely packed together, so that the influence of their irregular shapes and sizes became of preponderating importance, and masked the effects noted in the measurements by the alternative method. While the values of P gave an average of about 26 per cent., and showed, on the whole, comparatively little variation from this value, the values to be expected for p range from, say, 35 to 60 per cent., with an average of about 50 per cent.

Further experiments have been made with the Natal soils in order to examine more fully the relationship between P and p. The Haines shrinkage experiment was carried out on 33 of the soils from Table I. It was pointed out by Haines in his original paper that the method is most useful in the case of heavy soils, since the region of "uniform shrinkage" (*vide infra*) is very small in sandy soils. It was therefore decided that no useful purpose would be served by carrying out a complete set of measurements on the light Table Mountain Sandstone soils, and a selection of soils was made, with a bias in favour of the heavier soils. For the 33 soils used, therefore, the average clay content (29·1 per cent.) is higher than that for the complete series of soils. The results are given in Table V.

It was found that in this set of data, the average value of P ($\bar{P} = 35{\cdot}1$ per cent.) was considerably higher than that obtained for the 39 soils discussed in Paper I; hence, no direct comparison between

the two series is possible, but the results, so far as they go, give indirect support to the explanation outlined above. There is a fairly high correlation between P and p ($r_{Pp} = 0{\cdot}814$), but this is not as high as would be obtained if P and p were explicable in terms of identical soil features, and it appears very probable that the "tighter" the soils examined (*i.e.* the lower the value of P), the lower would be the correlation between P and p. Arguing from this standpoint, it is to be expected that the present data will show a closer correlation between p and C than between P and C, and this is found to be the case: $r_{pC} = 0{\cdot}771$, and $r_{PC} = 0{\cdot}642$. The significance of the correlation between P and C in the present case, as contrasted with the absence of such a relationship in the data of Paper I, is to be explained by the comparative "looseness" of the kneaded blocks now under discussion.

Table V.

Soil	C	S	p	P	σ	v	T
1	17·6	37·8	44·3	36·3	1·64	9·1	25·5
2	14·8	40·9	44·8	29·9	1·68	9·2	33·9
3	17·4	36·7	44·8	30·9	1·67	9·6	30·6
4	23·2	36·5	46·1	31·9	1·68	9·6	29·3
7	16·8	36·5	43·7	26·5	1·67	8·9	34·4
8	5·7	20·4	34·8	12·0	1·81	6·0	24·9
13	31·1	52·8	52·2	33·4	1·48	10·2	44·9
25	13·8	30·8	39·9	29·5	1·80	4·8	25·9
27	30·4	50·7	52·8	43·8	1·36	12·1	25·2
34	28·2	43·7	51·7	41·0	1·35	11·3	18·1
35	36·4	48·4	51·1	43·0	1·37	10·7	23·3
36	37·2	41·3	50·3	34·1	1·62	7·5	32·8
37	36·6	42·7	56·5	39·1	1·57	8·3	28·0
38	25·8	54·7	52·1	35·5	1·64	13·4	54·6
39	35·4	53·2	53·1	32·0	1·52	12·7	48·9
40	35·8	54·7	58·0	31·9	1·66	16·8	59·1
42	30·6	50·3	54·8	41·0	1·40	9·5	29·4
44	35·4	49·3	51·8	36·2	1·54	11·8	39·7
45	35·2	42·0	49·4	30·7	1·64	10·6	38·3
46	26·1	29·7	49·6	26·9	1·83	10·8	27·6
47	18·9	31·7	49·2	27·8	1·78	6·1	28·7
48	31·5	46·6	52·9	31·5	1·66	15·0	45·6
49	27·2	74·2	64·7	48·1	1·12	15·9	34·4
50	38·3	65·5	56·0	37·2	1·42	13·4	55·8
51	34·5	56·0	50·9	34·1	1·45	15·6	47·1
52	40·4	67·8	58·3	44·7	1·25	18·5	40·1
53	38·0	65·3	58·5	42·4	1·25	15·6	39·3
54	41·3	53·7	56·7	40·5	1·43	10·3	36·3
55	42·1	47·3	58·4	39·7	1·50	7·8	31·2
56	37·0	62·7	58·1	36·9	1·56	17·3	61·1
60	26·3	56·0	61·9	44·8	1·38	12·6	31·7
61	32·3	60·1	58·2	42·9	1·27	13·9	33·5
66	17·5	26·3	39·1	26·5	1·84	4·6	21·9

It should be added that a correlation between p and C on the complete set of 66 soils gave the value $r_{pC} = 0{\cdot}795$—a result in substantial agreement with the one quoted above for the 33 soils on which the

Haines experiment was performed. This last figure is lower than the one obtained in the corresponding case by Marchand ($r_{pC} = 0{\cdot}93$, on 106 samples), but it is undoubtedly significant. The general agreement with Marchand's results can also be demonstrated in another form. Marchand divides his soils into groups according to their types, and derives equations between p and C applicable to each group; these equations range between the extreme values

$$p = 0{\cdot}44\,C + 36{\cdot}8$$

and

$$p = 0{\cdot}61\,C + 32{\cdot}4.$$

It was not considered advisable to attempt such a detailed analysis with the results for the smaller number of soils examined here, but a single equation was developed to give the best fit for the whole of the data, irrespective of grouping into types. By plotting six point means of the p–C values, an eye estimate of the best line amongst these was made, and its equation then found to be

$$p = 0{\cdot}58\,C + 33{\cdot}5\text{[1]}.$$

This equation agrees satisfactorily with the general results expressed by Marchand's equations. For example, the "pore space at zero clay," which according to Marchand's equations should lie somewhere between 36·8 and 32·4 per cent., is given by this equation as 33·5 per cent.

(*e*) *Swelling and shrinkage of the soils.* The swelling and shrinkage experienced by a soil during wetting and drying are obviously factors of great importance from the point of view of the field behaviour of the soil, and various methods have been devised for estimating these effects[2]. As a general rule, heavy soils show greater swelling than light ones, and it was suggested tentatively by Keen and Raczkowski (*loc. cit.*) that there should be a close correlation between C and v, the swelling measured in the perforated box experiment[3]. Later work has provided only partial confirmation of this suggestion, however; Marchand (*loc. cit.*) calculated a correlation of 0·87 between C and v for ten groups of soils, but pointed out that the individual variations were very wide, and that two of the groups gave points very far from the best line representing the data for the other eight.

[1] The probable error of results calculated from this equation is $\pm 2{\cdot}7$. The regression line of p on C is $p = 0{\cdot}50\,C + 37{\cdot}1$; and that of C on p, $C = 1{\cdot}26\,p - 37{\cdot}2$, *i.e.* $p = 0{\cdot}79\,C + 29{\cdot}4$.

[2] See introductory paragraphs and references in Paper I.

[3] In the calculation of v, the specific gravity of the soil r (not r') should be used, since it is the "swollen" soil that is under consideration.

With the present data, also, the correlation is certainly significant, but not very high: $r_{Cv} = 0{\cdot}582$. If, however, attention is turned to the connection between v and S, or between v and I, better results are obtained: $r_{Iv} = 0{\cdot}817$ and $r_{Sv} = 0{\cdot}849$. These two results suggest that v gives a useful measure of the colloid status of the soil, in confirmation of the estimate derived from S, w and I; they therefore add an additional point in favour of the use of the perforated box experiment as a method of testing the nature of a soil.

The shrinkage of a soil on drying can be measured by Haines' method. In his shrinkage experiment, the shrinkage of the soil block may be divided into two parts—the region of uniform shrinkage, where the volume of the block decreases by the same amount as the volume of water lost by evaporation, and the region of residual shrinkage, where the change in volume is small compared with the volume of water lost by evaporation, since the pore spaces between the soil particles are no longer filled with water. Let V_0 be the volume and M_0 the mass of the oven-dried soil block, V the volume of the block when the moisture content is m per cent. by volume or n per cent. by weight, and σ the apparent specific gravity of the soil block. Then, in Haines' diagram, where V/V_0 is plotted against m/V_0, the curve for the region of uniform shrinkage is a straight line, and its equation is

$$\frac{100\,(V - V_0)}{V_0} = \frac{100m}{V_0} - P,$$

if P is expressed as a percentage of V_0, and the point $(V = V_0,\ m = 0)$ is taken as origin. Since $m = nM_0$, and $(M_0/V_0) = \sigma$,

$$\frac{100\,(V - V_0)}{V_0} = n\sigma - P.$$

Let T be the total percentage shrinkage of the block on drying from moisture content S; then

$$T = S\sigma - P,$$

and this is an expression from which T can be calculated from the results of Haines' experiment. (This calculation is, of course, equivalent to measuring the total shrinkage represented by the shrinkage curve extrapolated to moisture content S. For purposes of comparison with other data, this value of the moisture content appears to be the most suitable one to take for the initial point of the curve; it has already been shown that S or w represents the moisture content of a saturated soil.)

The total shrinkage calculated in this way is a quantity which might be used to give an estimate of the colloid status of a soil. Using the

data obtained in Paper I, it is found that T is moderately correlated with C: $r_{CT} = 0{\cdot}605$, and a corresponding result is obtained from the figures in Table V of the present paper; in this latter case, $r_{CT} = 0{\cdot}463$. One would not expect correlations of higher order than these, since the whole of the present work tends to show that the clay content of a soil is not such an accurate measure of the colloid status of a soil as has sometimes been suggested in earlier publications. The correlation between T and v from Table V is also moderate, $r_{Tv} = 0{\cdot}066$, and, again, a higher value could not be expected, in view of the complications arising from the close packing of the soil block.

5. Summary and Conclusions.

Experimental work has been carried out on a number of Natal soils, with the object of correlating their general type and field behaviour with their physical properties. The main results are set out below:

(1) Confirmation is obtained of the principal conclusions reached in the first paper of this series with regard to the physical significance of the sticky point, the loss on ignition, the clay content, and the water content in equilibrium with an atmosphere of 50 per cent. relative humidity. It is shown that the first and second and the third and fourth pairs of quantities respectively are closely correlated with one another, and that the first and second give the better estimate of the colloid status of the soil. The water content in equilibrium with an atmosphere of 50 per cent. relative humidity generally differs but little from the air dry moisture of the soil.

(2) The mechanical analyses of the soils fall into groups which correspond with the geological origins of the soils. This conclusion is probably to be explained by the immaturity of the soils on account of the prevailing topographical and climatic conditions. Work is now being carried out in order to investigate the bearing of this result on the profile formation of the soils, which should approximate to the podsol type.

(3) The significance of the soil constants determined by the Keen and Raczkowski perforated box experiment is re-examined. It is found that the same conclusions drawn with regard to the sticky point apply to the water retained by the saturated soil in the perforated box; that the pore space is closely correlated with the clay content of the soil; and that the volume swelling is closely correlated with the water retained, and, therefore, with the sticky point—so that the two factors, water retained and volume swelling, give a good estimate of the colloid

status of the soil. It is therefore suggested that the Keen-Raczkowski perforated box experiment might usefully be introduced as a preliminary measurement in testing the general nature of soils of unknown type. In cases where it is necessary only to determine whether there are significant differences between a large number of samples, however, the information can be obtained most easily from measurements of the sticky point (or loss on ignition) and the air dry moisture.

(4) An explanation is offered for the discrepancies in the results derived from the pore space of the soil as determined by the Keen-Raczkowski method and Haines method respectively.

(5) An expression is developed for the total shrinkage in the Haines experiment, and it is shown that this quantity gives a fairly satisfactory measure of the colloid status of the soil.

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FACTORS AFFECTING THE YIELD AND QUALITY OF MILK.

I. THE AGE OF THE COW.

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CONTENTS.

It is a well-known fact that dairy cows vary considerably in production according to age, though there is not absolute agreement as to the age which is most likely to be associated with maximum production. A large number of records have been collected by the Scottish Milk Records Association, and a start has been made at the West of Scotland Agricultural College to analyse some of these and see what can be learned from them. The data used will be discussed later, but in the meantime it may be mentioned that the records were obtained direct from the milk record books of the herd owners, and the writers wish to express their gratitude to the farmers concerned for the facilities they granted, as without their co-operation this study could not have been carried out. Credit is also due to Mr A. Paton and Mr A. M'Vicar, formerly of this Department, for the part they took in the preliminary work.

It was felt that before full use could be made of these records a study should be made of the variations due to the age of the animals, as it was not necessarily true that the age-correction factors obtained in other studies would apply to the records being used here.

I. HISTORICAL.

The study of the influence of the age of the cow on milk and butterfat production has been undertaken by a very considerable number of workers, though frequently with quite limited cattle populations. In some cases, also, short-time records, rather than the production for the complete lactation, have been used, and the results of such work, even at the best, are not of great value in indicating the producing ability of the cow.

In the majority of cases the animals used in the higher age classes constitute a selected population, but this point will be discussed later, as will the fact that frequently all animals used have had to reach some definite standard of production, and so poor producers have been entirely eliminated from the studies.

In some pieces of work on the variations which occur in production as the cows mature the age is not given, but only the number of the lactation, and for the present these will be neglected, as the data used here will be worked up later according to the order of the lactations as distinguished from age proper, and these reports will receive consideration at that time.

A. Influence on milk yield.

There have been great differences in the character of the data used to study the influence of age on milk production, so it is not always easy to compare the results obtained at different times. To begin with, the results from yearly records will be considered, and these will be followed by a review of the studies on records of shorter duration.

Table I. *Influence of age on yearly milk yield.*

Age of maximum yield Years	Ayrshire	Guernsey	Holstein-Friesian	Jersey
Over 5	Roberts(49) White and Judkins(62)	Beach(1) Roberts(49)	Roberts(49) White and Judkins(62)	Roberts(49) White and Judkins(62)
	—	White and Judkins(62)	—	—
6	—	—	Turner(59)	—
7	—	M'Candlish(39)	—	Hooper(33)
	—	Turner(59)	—	—
8	—	Gowen(19)	Gowen(22)	Gowen(19)
	—	Gowen(24)	Clark(8)	M'Candlish(39)
	—	Clark(8)	—	Turner(59)
	—	—	—	Clark(8)
9	Clark(8)	—	Gowen(19)	—
	Tocher(57)	—	—	—
10	Tocher(56)	—	—	—
11	Speir(55)	—	—	—
	Tocher(56)	—	—	—
13	Tocher(58)	—	—	—

The results of a number of studies on the influence of age on yearly milk production are summarised in Table I. In some sets of data all animals of 5 years of age or over have been grouped together and for these the age of maximum production can only be given as over 5 years, though this information is not of great value in the present study. The others give ages of maximum production ranging from 6 to 13 years, though the majority of them are around 7 or 8 years. So far as the individual breeds are concerned the majority of records apparently indicate that they all reach maximum production around 7 or 8 years, though there are records for both Ayrshires and Holstein-Friesians showing a higher age of maturity, and also one group showing the latter breed as maturing more rapidly than the others.

In addition to the records just set out there are results from other breeds, or from mixed data from two or more breeds. In these it is found that Shanks (52) says that production increases with age but gives no maximum, while Haas (27) gives 6 years, and Wing and Anderson (65), and Hills and Kibby (29) give 7 years as the age of greatest milk yield.

There are also a few writers who present no figures, but founding on their own or other data, give an age of maximum production. Among these are Kellner (35) and M'Connell (41), who give 7 years, and Lane (38), Ernst, Mohler and Eichhorn (15), and Wilson (63), who give 8 years as the age of largest milk production.

Short-time records have frequently been used in studying this problem. Some of these, taken from the early portion of the lactation, are perhaps of interest as indicating the age at which maximum daily production is attained, but they can hardly be presumed to give a true measure of the influence of age on total production for the lactation. On using 1-day Ayrshire records Tocher (57) found 7 years to be the age of maximum production, while Pearl and Miner (45) with 7-day records for the same breed obtained it at 11 years. The majority of studies of this type have been made on 7-day Holstein-Friesian records in the United States, and from these Wing and Anderson (64), Woll (66), and Beach (1) concluded that maximum production was reached somewhere beyond 5 years of age, while Miner (42) found that it was at 6 years, and Holdaway (31) at 7 years. With 7-day records for Jerseys, Pearl and Patterson (46) found maximum yield of milk at 8 years, while White and Drakeley (61) calculated it to be at 8 years for 2-day records of several breeds. Working with 8-month records for Jerseys, Gowen (20) ascertained that maximum production was at 7 years of age.

It can be seen that the results of studies of milk yields so far as they are influenced by age vary considerably, and this is no doubt very largely due to the factors already mentioned. On the whole, however, most sets of data show maximum production to be somewhere around 7 or 8 years of age. It was stated by Pearl (44) that milk flow increases with increasing age, but at a constantly diminishing rate (the increase in any given time being inversely proportional to the total amount of flow already attained) until a maximum flow is reached. After the age of maximum flow is passed the flow diminishes with advancing age and at an increasing rate. The rate of decrease after the maximum is, on the whole, much slower than the rate of increase preceding the maximum.

B. Influence on fat yield.

There are considerably fewer reports on the influence of age on the yield of butterfat than on its influence on milk yield, though there are sufficient to show the general tendencies. The reports on this problem are arranged in the same way as those on the variation in milk yield.

Neglecting those studies in which the records made above 5 years

of age are all put together, it is seen that the reports as to the age of maximum yearly fat production vary from 5 to 11 years, though they would appear to be centred around 7 to 8 years as shown in Table II.

Table II. *Influence of age on yearly fat yield.*

Age of maximum yield Years	Ayrshire	Guernsey	Holstein-Friesian	Jersey
Over 5	White and Judkins(62)	Beach(1)	White and Judkins(62)	White and Judkins(62)
	—	White and Judkins(62)	—	—
5	—	Graves and Fohrman(26)	—	—
6	—	—	Graves and Fohrman(26)	—
7	—	M'Candlish(39)	—	Hooper(33)
	—	—	—	Davidson(9)
8	—	—	—	M'Candlish(39)
	—	—	—	Davidson(9)
10	Tocher(56)	—	—	—
	Tocher(58)	—	—	—
11	Speir(55)	—	—	—

Of those who worked with cattle of several breeds, or with breeds other than those given in the table, Shanks (52) and Eckles (11) found that fat production increased with age but did not give a maximum, while Haas (27) and Wing and Anderson (65) state that maximum yearly production occurs at 7 years of age, and Brody, Ragsdale and Turner (7) found it to be at 8 years for both yearly and 10-month records. A number of workers have used 7-day records of Holstein-Friesians, and among them Brody, Ragsdale and Turner (7) give 8 years as the age of greatest fat production, while Wing and Anderson (64), Woll (66) and Beach (1) found it to be somewhere beyond 5 years.

These reports show much the same as was found in the case of milk yield—maximum production is somewhere around 7 or 8 years of age, and the same reservations regarding the selection of the data must be made. It is stated by Pearl (44) that, in general, the law he gave regarding the relationship of milk production to age is also true for the total fat yield.

C. Influence on fat percentage.

Of even greater controversial interest than the variations in the yields of milk and butterfat are the variations occurring in the percentage of fat in milk. Owing to the importance of the question from a legal standpoint it has received a considerable amount of attention. The majority of the results available on yearly records of the four dairy breeds being considered here are given in Table III.

Table III. *Reports showing a decrease of average yearly fat percentage with age.*

Ayrshire	Guernsey	Holstein-Friesian	Jersey
Högström(30)	Roberts(49)	Roberts(49)	Roberts(49)
Speir(55)	Gowen(20)	Gowen(22)	Gowen(21)
Roberts(49)	M'Candlish(39)	—	M'Candlish(39)
Tocher(56)	—	—	—
Tocher(57)	—	—	—
Tocher(58)	—	—	—

All of the reports for yearly records of the four breeds included in Table III show that the percentage of butterfat in milk tends to decrease as the cows advance in age, though in the Jersey records reported on by M'Candlish (39) there was a preliminary increase to 4 years of age, followed by a decrease for succeeding years, while Tocher (58) found with Ayrshires that there was an increase after 11 years of age. Both M'Connell (41) and La Cour (37) state that the fat percentage declines with age.

It was stated by Kellner (35), while considering the problem in general, that there were no appreciable changes in fat percentage with age, and Hooper (33), when working with Jerseys, came to the same conclusion, as did Eckles (12), also, after summarising many thousands of records from various breeds.

When short-time records are considered it is found that Pearl and Miner (45) with 7-day Ayrshire records, Tocher (57) with 1-day Ayrshire records, Woll (66) and Hooper (32) with 7-day Holstein-Friesian records, and White and Drakeley (61) with 2-day records of several breeds, all reported a decrease in fat percentage with age, though Tocher (57) found a preliminary rise to 5 years, and White and Drakeley (61) state that it is high at 3 to 5 years. On the other hand, Wing and Anderson (64) found no appreciable change in fat percentage with age in 7-day Holstein-Friesian records.

The one marked exception to the reports quoted is that of Ernst, Mohler and Eichhorn (15), who state that fat content rises with age, though they give no evidence to prove it.

On the whole the evidence goes to show that the highest fat percentage is obtained with young cows, and then there is a fall which may not be very marked for a time as the fat content remains fairly steady from year to year during the period of greatest usefulness of the cow; then, in later life, there is a fall which may be of significance. One or two cases appear to show that with some of the breeds the fat percentage may remain constant, or even rise, for the first two or three

years of the productive life of the cow, instead of falling as is generally believed to be the case; but this point needs further investigation.

D. Other influences.

It was reported by Klein and Kirsten (36) that abnormalities occurred in the Reichert Meissel and iodine values of butterfat from an aged cow, and they stated that good butter could not be made from the milk of old cows. On examining the milk of 46 cows Hills and Kibby (29) found that in the majority of cases the solids-not-fat tended downwards with advancing age, though in a few cases the change was in the opposite direction, and some showed no change with age, while Ernst, Mohler and Eichhorn (15) also state that the solids-not-fat fall with advancing age.

It was reported by Eckles and Palmer (13) that neither the chemical composition of the milk, nor the physical and chemical constants of milk fat, of aged cows show any abnormalities which may be attributed to age, while butter made from the milk of a 19-year-old cow in her thirteenth lactation was of excellent quality and kept well.

After examining a large number of samples Tocher (57) came to the conclusion that the percentage of ash and total nitrogen, and the freezing point, are not altered appreciably by the age of the cow, while the percentage of lactose, casein, and solids-not-fat, as well as the refractive index and specific gravity fall with age. On the other hand, the percentage of albumen rises gradually to 10 years of age and then falls gradually. It is stated by White and Drakeley (61) that the percentage of solids-not-fat decreases continuously with age, though in some cases the decrease is almost imperceptible during the earlier lactations, while the actual weight of solids-not-fat increases until about 8 years of age.

There is not as much information available on the percentage of solids-not-fat as there is on the yield of milk and percentage of butterfat. However, it would appear that there is a decrease in solids-not-fat with advancing age, though the change may at times be not very marked.

E. Summary of work on Ayrshire yearly records.

Owing to the methods of collecting the data a considerable number of the pieces of work reported are not strictly comparable with the records to be considered here, but the reports by Speir (55) and Tocher (56, 58), require further attention as they are based on yearly records of Ayrshire cows collected by the Scottish Milk Records Association.

In these records there is a gradual increase in milk production from

2 to 11 years of age in the case of the work of Speir (55) and then irregular variations with a downward tendency, while in the first report of Tocher (56) the milk yield is shown to rise to 10 years of age in one set of records and to 11 years in the other, and after the maximum is reached there is a general fall in production. In the second report of Tocher (58) the age of maximum production is given as 13 years. The total fat production shows similar variations in the three reports, reaching a maximum at 10 or 11 years of age. The variations in the fat percentage are in the opposite direction and would appear as a general rule to fall as the yields of milk and butterfat approach a maximum and then rise with the final decline in total yield.

The records on which these reports are based are all from the same source and give similar results, but have one drawback in common—the animals in the higher age classes constitute a selected population, while in the lower age classes there is an unselected population. In the younger classes a mixed group of cows, good, poor and indifferent, occur, but the poor producers are weeded out at an early age and so only relatively high producing individuals are left in the older age classes. As a consequence the production of good cows in the higher classes is compared with the production for the average run of individuals in the lower classes, and so the data collected in this way show a relatively higher production at the older ages than is justified.

II. THE DATA.

As has already been mentioned, a study is being made of the Scottish milk records, and to begin with it was decided to take some of the records obtained in the first 20 years of recording work in Scotland, from 1903 to 1922 inclusive. In this period 303,075 records of cows were obtained. The records from three counties, in which practically half of all Scottish milk recording has been conducted, Dumfries, Kirkcudbright and Wigtown, have been used, and only the data from societies which kept complete yearly records have been included in this study. It was felt best to neglect the earlier records of some of the societies where records were not kept for the full year. Some of those dropped from this work were almost continuous, but it was deemed advisable to drop all that were not continuous rather than to attempt to put an arbitrary time limit on the records.

Then again it was necessary to limit the investigation to registered cows as it was only in this way that it was possible to trace the records

of an individual cow from year to year with certainty, and only such records could be of value in the study of breeding and similar factors. As a consequence, the only records used were those of cows entered in the herd books of the Ayrshire Cattle Herd Book Society, or appendices thereto. These necessary limitations have considerably reduced the number of records available for study.

Though milk recording started in the three counties in 1903, it was not until 1906 that it was carried on from year to year, and not until 1912 that it was continuous throughout the year. As a consequence, of the 149,833 milk records obtained in the counties in the 20-year period, only 71,578 could be used. Then, again, only 22,714 of these records were for Ayrshire cows the breeding of which could be traced from the record books.

It now becomes necessary to consider which of the records available are suitable for the present study. As has already been mentioned much of the earlier work on the influence of age on production is not as valuable as it might be on account of the fact that the great majority of the animals in the higher age classes are really selected individuals, kept because of their producing ability, and comparing these with the general run of cows at younger ages does not give a true conception of the variations in production due to age. Consequently it was decided to use only cows with known production records for five or more consecutive lactations, beginning with the lactation following the first calving. The cows were then grouped according to the number of lactations available —5, 6, and so on up to 11 lactations, the highest number found in the records used.

The lactations for each cow were arranged according to the age of the cow at the time of calving, and it was necessary to make age classes which would be suitable. In the south-west of Scotland it is the custom to bring heifers into the producing herd at about 2½ years of age, and so the first age class was taken as 2½ to 3½ years of age. There are a few animals calving at a little under 2½ years, and an occasional one considerably earlier, but as in practice all such animals are generally considered together they have been included in one group, that of animals under 3½ years of age. For convenience this may be considered as the 3-year-old group, then all between 3½ and 4½ years of age are put in the 4-year-old group, and so on.

In a few cases it was found that an animal had two calvings within the age year, and there were also cases where more than a year elapsed between calvings. Three methods of handling such records were possible:

discard them; put the records in the corresponding lactations irrespective of age; or where necessary put two records in one age class, and in the other cases have one age class without a record. As it had been determined to use age classes the last method was adopted, and so the number of animals in any age class does not necessarily correspond with the number of cows used.

It was decided to permit one of either, or each, of these irregularities to be included in records which were otherwise suitable. The number of animals and the number of lactations finally used are given in Table IV.

Table IV. *Cows and lactations used.*

Age class in years		3	4	5	6	7	8	9	10	11	12	13	
Group	No. of cows	Number of lactations											Total
A	339	361	313	322	320	340	—	—	—	—	—	—	1656
B	177	193	163	170	168	166	177	—	—	—	—	—	1037
C	107	117	98	101	98	103	104	108	—	—	—	—	729
D	64	67	64	57	63	61	63	60	64	—	—	—	499
E	39	42	37	37	37	37	38	39	38	39	—	—	344
F	10	10	8	10	8	10	10	10	10	8	10	—	94
G	2	2	2	2	2	2	2	2	2	2	1	2	21
Total	738	792	685	699	696	719	394	219	114	49	11	2	4380

For convenience the various groups of cows are lettered, *A* with records from 3 to 7 years, *B* from 3 to 8 years, *C* from 3 to 9 years, *D* from 3 to 10 years, *E* from 3 to 11 years, *F* from 3 to 12 years, and *G* from 3 to 13 years of age.

The total number of animals ultimately used was 738 with 4380 lactations.. Naturally the group with records only to 7 years of age, that is with 5 lactations, was the largest, including 339 cows, while the numbers gradually decreased until the group with records up to 13 years of age, or with 11 lactations, was reached, and in it there were only 2 cows.

III. RESULTS OBTAINED.

The average milk and butterfat production and average fat percentage for each group and age is given in Tables V, VI and VII.

A. MILK YIELD.

If the milk production be considered first it is found that the group with records from 3 to 7 years of age increases in production to 7 years, while the groups with records from 3 to 8 years and from 3 to 9 years increase in production to 7 years of age and then show a slight decline. The groups following are more irregular, very probably on account of

the smaller numbers of animals available. The 3 to 10 years group rises in production to 5 years, falls off slightly at 6 years, and then keeps rising to 9 years, with a fall again at 10 years of age. The 3 to 11 years group rises quickly to a relatively high point at 5 years, falls to 8 years, rises to a maximum at 10 years, and falls off a little at 11 years of age, while the 3 to 12 years group rises to a maximum at 6 years, falls steadily to 11 years, and then shows a final rise at 12 years of age. The 3 to 13 years group rises to 7 years, falls at 8 years, rises to 11 years with a maximum at that point, falls at 12 years and rises again at 13 years.

Table V. *Average milk production, in pounds.*

Age in years / Group	3	4	5	6	7	8	9	10	11	12	13	Average
A	6220	6467	6963	7183	7287	—	—	—	—	—	—	6816
B	6344	6561	6946	7357	7533	7422	—	—	—	—	—	7015
C	6956	6765	7002	7132	7868	7612	7549	—	—	—	—	7271
D	6743	7020	7177	7152	7215	7723	7919	7,272	—	—	—	7270
E	6238	6925	7695	7511	7163	7075	7702	7,804	7,732	—	—	7306
F	6415	7048	8705	8903	8578	8382	8389	7,312	6,774	7786	—	7845
G	7600	8035	8315	9070	9580	8085	8920	10,685	10,835	9030	10,095	9118
Average	6407	6619	7049	7257	7439	7514	7722	7,429	7,703	7899	10,095	7061

Table VI. *Average fat production, in pounds.*

Age in years / Group	3	4	5	6	7	8	9	10	11	12	13	Average
A	240·6	248·0	263·5	267·2	272·6	—	—	—	—	—	—	258·2
B	246·2	243·6	263·4	278·7	282·0	278·7	—	—	—	—	—	265·1
C	270·5	254·7	263·3	270·0	304·3	288·8	282·2	—	—	—	—	276·4
D	258·5	266·1	269·6	266·6	267·3	288·7	296·0	272·7	—	—	—	273·0
E	240·5	253·8	280·4	268·2	253·6	265·9	283·6	286·9	286·9	—	—	269·5
F	252·5	272·3	329·1	334·5	319·9	317·6	315·4	272·5	254·3	250·4	—	292·2
G	302·1	270·7	293·5	321·7	342·9	293·2	340·3	398·5	391·6	303·2	348·1	328·9
Average	248·2	248·7	265·9	271·6	279·3	282·8	288·2	279·5	285·9	255·2	348·1	266·3

Table VII. *Average fat percentages.*

Age in years / Group	3	4	5	6	7	8	9	10	11	12	13	Average
A	3·87	3·83	3·79	3·72	3·74	—	—	—	—	—	—	3·79
B	3·88	3·71	3·79	3·79	3·74	3·76	—	—	—	—	—	3·78
C	3·89	3·77	3·76	3·79	3·87	3·79	3·74	—	—	—	—	3·80
D	3·83	3·79	3·76	3·73	3·70	3·74	3·74	3·75	—	—	—	3·76
E	3·86	3·66	3·64	3·66	3·54	3·76	3·68	3·68	3·71	—	—	3·69
F	3·94	3·86	3·78	3·76	3·73	3·79	3·76	3·73	3·75	3·22	—	3·72
G	3·97	3·37	3·52	3·55	3·58	3·63	3·82	3·73	3·61	3·36	3·45	3·61
Average	3·87	3·76	3·77	3·74	3·75	3·76	3·73	3·76	3·71	3·23	3·45	3·77

If all groups be now taken together it is found that, on the average, the production of milk increases to 9 years of age and is then very irregular. The increase after 7 years of age, however, is very small and

of no practical significance, and at that it is more apparent than real as the numbers of animals in the higher age classes are too few to give results of much value.

B. Fat yield.

The butterfat yields go in somewhat the same manner as the milk yields, though on the average there is little change from 3 to 4 years of age. The 3 to 7 years group rises to a maximum at 7 years, while the 3 to 8 years and 3 to 9 years groups increase in production to 7 years and then fall off slightly. The 3 to 10 years group rises to 5 years, falls off slightly at 6 years, and then rises to 9 years with a slight fall at 10 years. The 3 to 11 years group rises rapidly to a relatively high point at 5 years, falls to 7 years, and then keeps rising to a maximum at 11 years. The 3 to 12 years group rises to a maximum at 6 years, falls at 7 years, rises again at 8 years to a slight extent, and then falls to 11 years with a final slight rise at 12 years of age. The 3 to 13 years group falls slightly at 4 years, rises to 7 years, falls at 8 years, rises to 10 years, falls to 12 years and shows a final rise at 13 years of age.

When all the groups are combined it is found that butterfat production follows much the same course as the milk yield. It increases to 9 years of age and then is very irregular, though the increase after 7 years of age is not of much significance.

C. Fat percentage.

Coming now to the percentage of butterfat present in the milk it is found that there are no such regular changes from age to age in the various groups as were found in the case of the milk and butterfat yields. However, in every group it is found that the percentage of fat at 3 years of age is higher than at any other age. If all the groups be combined the maximum fat percentage is obtained at 3 years of age, there is a considerable drop at 4 years, and then the fat percentage remains fairly constant to 7 or 8 years of age and drops off to a certain extent in the higher ages.

D. Age relationship.

The relationship existing between the yields at various ages can be shown by expressing them as percentages of the production at any given age. Consequently the production of milk at 7 years of age has been taken as 100 for each group, and the production at other ages within the group expressed as a percentage of it, and the results are given in Table VIII.

Table VIII. *Milk production as percentage of production at 7 years of age.*

Age in years / Group	3	4	5	6	7	8	9	10	11	12	13	Average
A	85	89	96	98	100	—	—	—	—	—	—	94
B	84	87	92	98	100	99	—	—	—	—	—	93
C	88	86	89	90	100	97	96	—	—	—	—	92
D	93	97	99	99	100	107	110	101	—	—	—	101
E	87	97	107	105	100	99	108	109	108	—	—	102
F	75	82	101	104	100	98	98	85	79	91	—	92
G	79	84	87	95	100	84	93	112	113	94	105	95
Average	86	89	95	98	100	101	104	100	104	107	135	95

The average production at each age, calculated as a percentage of the production at 7 years of age, shows what has already been demonstrated—there is an increase in milk yield as the cow matures, and for the majority of the groups where there are fair numbers of animals it reaches a maximum at 7 years of age, while later there are irregular variations. The same holds true for the yield of fat, but the fat percentage, on the other hand, is highest in the 3-year-old records, remains fairly constant from 4 to 8 years of age, and then shows a slight downward tendency.

IV. DISCUSSION OF RESULTS.

Now that the changes occurring with age have been considered it remains to discuss the causes of the changes, as well as the use of the data in the interpretation of milk records. In addition certain limitations to the data and the relationship of age variations in production to practical problems need attention.

A. Limitations to data.

In the study of many problems it sometimes occurs that the data available are not as extensive as is desirable, or have other limitations. This has frequently been the case with the present problem. Many of the earlier studies were subject to quite serious limitations in their data, and, though some of these have been eliminated as far as possible in the data on which the present study is based, there still remain certain limiting factors which should not be overlooked.

1. *Short-time records.*

A very large number of the studies of the influence of age on milk and butterfat production, especially in the United States of America, are based on short-time records. These short-time records are generally taken

early in the lactation period and usually have a duration of 7 days. They may show the ability of the cow for production during a limited period, but, in addition to high initial production, persistency of production is essential before there can be a large yield for the lactation as a whole.

The yield of a cow for a short period at the beginning of the lactation may give information of interest, but it does not give a true indication of her real value, as it has been shown by M'Candlish, Gillette and Kildee (40) and Sanders (51) that persistency of production is just as important as high initial production in determining the ultimate yield for the lactation. Consequently it is much better in determining the influence on production of factors such as age to use lactation period records rather than records for a short time at the beginning of the lactation, though the latter may aid in the interpretation of the longer records.

Even when these short-time records are left out of consideration, however, there are large numbers of records for various breeds available, and these have been relied upon to a great extent in reviewing the work done on this problem.

2. *Variations due to selection.*

A factor which may have a marked influence on the value of data concerned with the influence of age on the production of dairy cows is selection, and there are two principal ways in which it can operate, and which, for the present purpose, may be termed conscious and unconscious selection.

(*a*) *Conscious selection.* The great majority of reports on the influence of age on production are based on studies of records published by the dairy cattle breed societies in the United States of America. To obtain entrance to these published lists a cow must have reached some definite standard of production, and so the studies do not include any really poor producers. For inclusion in these lists the standard of production demanded is usually around 250 lb. of butterfat for a cow 5 years old or over, while younger animals have a smaller entrance requirement. These lists contain only cows which have been selected because of their producing ability, and though there is no evidence to show that the change in production due to age varies with cows of unequal producing powers, yet it cannot be presumed that the rate of change is necessarily the same for all classes of cows no matter what their producing ability.

In the present study all cows which met the requirements in other ways were retained irrespective of their producing ability, and it is

hoped at a later date to classify them according to their production and determine if there is any variation in the rate of change with age in high and low producers.

(*b*) *Unconscious selection.* Any dairy cattle population is always being depleted through death, disease and other influences, and in addition even in poorly managed herds the low producers are always being eliminated. As a consequence of this latter fact the older age classes will contain a higher percentage of naturally good producers than is to be found in the younger classes, and so there may be a tendency for the older classes to show a relatively higher production than they should.

In all previous reports this point has been neglected. In the present study, as has already been pointed out, only cows which had records for five or more successive lactations were used, and so the influence of this so-called unconscious selection is eliminated to a certain extent. However, it must be admitted that poor producers will, and should, be eliminated from a herd as soon as possible, and consequently the data used in this study is subject to some extent to the last criticism just offered regarding previous work.

(*c*) *Conscious and unconscious selection.* Some information regarding the interplay of the two types of selection and their relationship to the variations in production due to the ageing of the animals can be obtained from a comparison of the results in this and previous studies. The great majority of earlier studies give the age of maximum milk and butterfat production as around 7 to 8 years, though some run as high as 13 years for milk yield and 11 years for fat production.

If these reports be divided on the basis of whether or not they were subject to systematic selection or elimination by natural causes, it will be found that all the reports giving an age of maximum production above 8 years are, with the exception of that by Gowen (19), subject to unconscious selection to a much greater degree than those giving a lower age for maximum yield. In the present report both forms of selection have been reduced to some extent, though neither of them could be completely eliminated, and in comparing the results of the present study with those of earlier pieces of work it is seen that the present report agrees fairly closely with those earlier studies which were subject to systematic selection to a greater degree than to the other type of selection, as the majority of these, like the present study, give 7 or 8 years as the age of maximum production.

This would indicate that conscious selection does not alter the value of the results to any considerable degree. In other words, there is a

probability that the rate of maturity is much the same among good as among poor producers, though this statement must be accepted with caution since, though conscious selection was less obvious in the present study than in the earlier ones, yet it did occur.

Elimination by natural causes played a more important part in influencing the results of some of the earlier studies than did systematic selection. The studies by Speir (55), Tocher (56), Clark (8) and Tocher (57, 58) were affected by the former and all give the age of maximum production as higher than that given in other studies. This is simply due to the fact that these studies include a random population in their earlier ages, while in the higher ages the animals constitute a selected group since the majority of them have been retained on account of their producing ability.

The results of Speir (55) and Tocher (56, 58) may be compared directly with the present study as the data are from the same source. In both these earlier studies large numbers of animals were used and in the younger ages a considerable number of relatively poor individuals were included, while these had been partially eliminated from the older age classes by selection on the part of the farmers. As a consequence the animals of the older ages showed relatively greater production of both milk and butterfat than did the younger animals, and so gave ages of maximum milk production of from 9 to 13 years, and of maximum fat production of from 10 to 11 years, while in the present study with data from the same source maximum milk and butterfat production were found to occur around 7 to 8 years of age.

B. BREED VARIATIONS.

A considerable number of reports are available on the influence of age on production in the Ayrshire, Guernsey, Holstein-Friesian and Jersey breeds, and with these the relative rates of maturity can be studied.

1. *In milk and fat yield.*

It can be seen that animals of the Guernsey, Holstein-Friesian and Jersey breeds reach maturity at about the same age, while the majority of previous reports would appear to indicate that the Ayrshire is longer in reaching maturity than the other breeds. However, if the data where all animals of 5 years of age or over are grouped together be left out of consideration, it is found that the only reports on Ayrshires then available are those which are influenced by unconscious selection.

It has already been indicated that these give an unduly high age of maximum production, and the data on which the present report is based indicate that the Ayrshire breed reaches highest production at 7 to 8 years of age. As a consequence, it may be taken that in general the four dairy breeds mature at about the same rate so far as milk and butterfat production are concerned.

2. *In fat percentage.*

The bulk of the evidence goes to show that with all the breeds on which data are available the fat percentage tends to decline with age. There are, however, indications in the reports of Eckles and Palmer (13), Hooper (33) and M'Candlish (39) that in the Jersey breed there may be a rise in fat percentage during the first two or three lactations, with a subsequent fall. This point needs further investigation.

C. Practical considerations.

In this study a large number of records are available from a considerable number of farms in the south-west of Scotland, and as these records were spread over a period of 20 years they should give some results of practical significance. Cows that were eliminated from the herds at an early age, because of low production or other factors, are not included in the study, and so the results should indicate what is to be expected with the cow of fair or good production, though it does not give any information regarding the development of the poor cow. This is not really wanted for practical purposes, however, as the dairy industry is better without the poor cow. A few facts of interest appear to be brought out fairly clearly.

1. *Average production.*

The average production of all cows at all ages in this study was 7061 lb. of milk of 3·77 per cent. butterfat content, or a yield of 266·3 lb. of butterfat. This must be looked on as very satisfactory, especially in view of the fact that many of the records included were made during the years 1914 to 1919 when labour was at a premium and concentrates scarce and expensive. These two factors undoubtedly tended to limit the production of all cows in the south-west of Scotland to a certain extent.

Under the Scottish Milk Records Association all cows producing over 250 lb. of butterfat per year are listed as Class I cows, while heifers with their first calf giving 200 lb. of fat are put in this group. All animals

giving less than two-thirds of these requirements are put in Class III, while the remainder are in Class II.

This method of classification was adopted in 1914, and, in that year, out of a total of 26,424 cows tested 39½ per cent. were in Class I and 9 per cent. in Class III. In 1927, the last year for which records are available at present, 29,459 cows were tested and the percentage of Class I animals had increased to 65¾, while that of Class III animals had been reduced to 2¼. This was in spite of the fact that each year a number of untested herds came into the Association. These facts all go to show that a high level of production is maintained in the dairy herds of Scotland and that the Milk Records Association has been of untold benefit to the industry.

2. *Most productive age.*

Ayrshire cows in Scotland evidently mature at about 7 years of age, that is at about the same age as the other dairy breeds. As a general rule therefore an Ayrshire cow may be expected to be at her best at around 7 to 8 years of age, though in some cases greatest production may be reached a little earlier or a little later. This fact requires considerable attention. The average life of a cow in a dairy herd is around 4 years and so it is evident that a very large percentage of the cows are removed from the herd before they reach the age of greatest usefulness. Granted that a considerable number are removed as poor producers, and that a big turnover in the animals within a dairy herd is probably inevitable, yet every possible effort should be made to increase the percentage of animals that remain in the herd until maturity is reached. In this way the average production could be raised appreciably and considerable economies effected.

3. *Productive life of the cow.*

In the great majority of the groups into which the animals have been divided, and especially those where there are a considerable number of animals, maximum production of milk and fat is obtained around 7 years of age. There is a fairly regular increase up to this time, while above it the production shows a tendency to decline gradually, though the changes are irregular and not large as a rule, and there are a few large irregular changes where only a few animals are included in a group.

From 7 years of age onwards to 9, 10 and even 11 years of age the level of milk and butterfat production is fairly uniformly maintained,

thus indicating that when the cow has reached maturity she does not necessarily deteriorate rapidly in production. This again shows that everything possible should be done towards keeping mature cows actively producing in the herd, as it is from these that the greatest production is to be obtained.

There is another aspect to this problem, however. It would appear that many of the animals in the higher age groups have perhaps been kept in the herds because they were relatively good producers, rather than being relatively high producers because they were kept in the herds. This can be appreciated if the records for the various groups be studied.

If the average record of the 3 to 7 years group be compared with that for the 3 to 8 years group, and so on, as is done in Tables V and VI, it will be found that the average for the group rises with the number of lactations completed. This may be brought about by two factors and is probably due in part to both. First, the higher groups include relatively more records produced at or beyond maturity than do the lower age groups, and as production increases with age up to a certain point this would tend to increase the averages for the groups retained to the higher ages. Then, again, the animals kept to the higher ages are perhaps naturally good producers, otherwise they would not have been retained, and so the averages for the higher groups are increased.

This can be tested in another way. Consider the 3-year-old records for each group, the 4-year-old records for each group, and so on. It will be found that there is a tendency for the record for any given age to rise as the groups kept to the greater ages are reached. This is not absolutely definite all the way through, but the tendency is noticeable and would perhaps be still more pronounced if larger groups of animals were available.

It is probably safe to conclude therefore that not only has the dairy cow a considerable period of usefulness after she reaches maturity, but also that there is a tendency to retain good producing cows in the herd even longer than other individuals. This latter practice will be justified owing to the breeding value of the good producers. It will pay to retain them even after they begin to decline in production if they are still capable of breeding.

4. *Age and the law.*

The law is not interested in the yield of milk or butterfat produced by dairy cows, but it does pay marked attention to the fat percentage of the milk, as all milk offered for sale for human consumption must

contain at least 3 per cent. of butterfat. The fat percentage in the milk of the animals studied here shows on the whole less variation with age than do the yields of milk and butterfat. It is highest in the 3-year-old form, drops considerably in the next, remains fairly constant until shortly after maturity is reached, and then shows a downward tendency.

The 3-year-old fat percentage is significantly higher than that for other ages, averaging 3·87 per cent. for all groups. The fall to 3·76 per cent. in the 4-year-old form is quite marked, then from 5 to 8 years it is fairly steady, never going higher than 3·77 per cent. or lower than 3·74 per cent. After this there is a downward tendency, however, though it is not very marked until the ages of 12 and 13 years are reached, when the fat contents are 3·23 per cent. and 3·45 per cent.

In the ordinary range of ages, up to 8 or 9 years, there is therefore little risk of a fall which may be attributable to age large enough to bring the fat percentage below the legal limit in the ordinary run of cases. However, when advanced ages are reached the fall may be quite marked, as is seen with the two higher groups. With cows which in their earlier years do not give a very high percentage of butterfat there is a risk that at advanced ages they may give milk near to, or below, the danger point so far as fat percentage is concerned, and in the same way cows normally testing well at an early age might at a greater age give very low testing milk if some other detrimental factor were also at work. The risk of old cows being below the presumptive standard in butterfat should therefore not be neglected.

5. *Is milk recording fair to the heifer?*

The Scottish Milk Records Association demands that a cow produce not less than 250 lb. of butterfat, or, as it is stated, 2500 gallons of milk of 1 per cent. of fat, before she can enter Class I, while the required record for a heifer with her first calf is 200 lb. of butterfat. Is the handicap fair?

There has been set out in Table IX the required butterfat production for each age from 3 to 7 years inclusive, as well as the average production for all animals of these ages in the present study. Then from the actual production at the various ages and the standard requirements for mature production at 7 years of age have been calculated fair entrance requirements of animals up to 7 years of age.

It is evident from the results in this table that animals in 3-year-old form have to meet quite reasonable requirements to get into Class I as the average production for this age exceeds the requirements by 48·2 lb.

of butterfat, or 24 per cent., while mature 7-year-old animals on the average exceed their requirements by only 29·3 lb. of fat, or 12 per cent. To be put to a test equal to that applied to mature cows the 3-year-olds should have an entrance requirement of 222 lb. of fat. The animals at 5 and 6 years of age also have a fair margin of safety, though it is not as large as that for the heifers and mature cows.

The 4-year-old class, however, is labouring under a severe handicap. Here the average production of the group is 1·3 lb., or 1 per cent., below the production called for to obtain entrance to Class I. It is thus evident that a considerable number of 4-year-olds, which in view of their production when compared with other ages should be entered in Class I, are excluded therefrom. Consequently, in order that they may be put on an equality with other ages, the entrance requirements for 4-year-olds should be lowered to 223 lb. of fat.

Table IX. *Comparison of required and actual production of butterfat in Scottish Milk Records Association.*

Age (years)	Required production (lb.)	Average actual production (lb.)	Production above requirements (%)	Equivalent production (lb.)
3	200	248·2	24	222
4	250	248·7	−1	223
5	250	265·9	6	238
6	250	271·6	9	243
7	250	279·3	12	250

To give animals of all ages equal justice an adjustment in the entrance requirements to Class I should be made. In doing this a slight advantage should still perhaps be given to the 3-year-olds, since this class, as defined in the present study and in practice, contains some very young individuals. Therefore, instead of calling for 222 lb. of butterfat, which corresponds directly to an entrance requirement of 250 lb. for mature animals, it could be set at 210 lb. Then a fair increase in the requirements for each added year of age would be 10 lb. of fat, so that the requirements for other ages would be 220 lb. for 4-year-olds, 230 lb. for 5-year-olds, 240 lb. for 6-year-olds, and 250 lb. for animals 7 years old or over.

6. *Selection for fat percentage.*

When the average 3-year-old records, the average 4-year-old records, and so on, for each group were considered in so far as the milk production was concerned, it was found that there was a tendency for the record at any given age to rise as the groups kept to the higher ages were reached.

This indicates that there has been some selection on the basis of milk yield—in other words, there has been a tendency to keep in the herds the animals with the largest milk yields.

If the butterfat production be considered in the same way the results are somewhat the same, though the butterfat yields do not increase as markedly as the milk yields. This is probably due to the fact that less attention has been given to selection on the basis of butterfat yield, or butterfat content, than to selection for milk production. It is undoubtedly true that in the selection which does take place in many herds in the south-west of Scotland more attention is paid to milk yield than to fat yield or fat percentage, though the latter does at times receive some consideration.

Where milk is being sold by the gallon, irrespective of its butterfat content so long as it complies with legal requirements, there is probably little justification, from the commercial standpoint, for paying much attention to selection on the butterfat basis. However, in view of the effort to popularise milk of high quality, more attention should be given to the problem. Then again, where milk is sold on the basis of butterfat content, or is made into cheese, there should be selection for butterfat as well as for total milk yield, since milk of increased butterfat content will bring in greater monetary returns.

When all the available records are considered it is found that the 3-year-olds show a significantly higher butterfat percentage than the others. This is of importance when cows are being selected on the basis of the butterfat content of their milk. Sometimes a young animal giving milk with a low fat percentage is retained in the herd in the hope that her fat percentage will increase with age in later lactations. This is not justified, as the fat percentage at 3 years of age is higher than at any other age. Provided the heifer has had a fair opportunity so far as feeding and management are concerned she will produce milk of a higher fat percentage in her first lactation than at any time in later life under the same conditions, so if she cannot produce a reasonable fat percentage in her first lactation she need not be expected to do so later.

7. *Changes in yield.*

The results of this study again emphasise a fact very frequently found to hold true in other connections, but not always receiving the attention it merits. As the milk yield changes the butterfat yield also changes, in the same direction but at a slower rate, and as a consequence the fat percentage changes in the opposite direction.

D. Correction factors for age.

One of the objects of this study was to arrive at correction factors for age which could be used in the further study of records obtained in the south-west of Scotland. The tendency in the data presented here is for maximum or mature production to be reached about 7 years of age, and after that age there is very little variation in production for a considerable time, and the changes which occur are irregular. Consequently it is felt that it is not necessary to apply a correction factor to the records beyond 7 years, provided the production at that age be considered as the level to which the earlier records are corrected.

Table X. *Correction factors for age.*

Age in years	Milk yield	Fat yield
3	1·16	1·13
4	1·12	1·12
5	1·06	1·05
6	1·03	1·03
7	1·00	1·00

The records below 7 years show fairly consistent variations and correction factors have been worked out for both milk and fat production. These are given in Table X and have been obtained by expressing the production at 7 years as a percentage of the production at each of the earlier ages. Thus the average milk yield at 7 years of age, 7439 lb., is 116 per cent. of the average production at 3 years, 6407 lb. Consequently the 3-year-old production of milk can be corrected to the maturity level of 7 years by multiplying by 1·16.

E. Causes of variation with age.

The causes of the variations in production with age are perhaps not quite as simple as they may at first appear. They are frequently attributed to the fact that the cow is maturing but this does not explain them. The problem of the maturity of the cow is one that needs special attention along the lines of growth and development, while at the same time certain of the components of lactation production, such as initial yield and the rate of decline, cannot be neglected.

1. *Maturity.*

There are two factors which appear to be intimately associated with the maturity of the cow and with variations in milk production. In the term growth are generally included changes in body size and weight, and with these there is probably associated an increase in the active secretory tissue of the udder; while by development is indicated the

increased functional activity of this secretory tissue, as well as other parts of the body, through use. Though these terms are not synonymous it is not easy to separate them as they are very intimately connected.

(a) *Growth.* There are a few pieces of work on growth which have some bearing on the variations in production with age, and it is interesting to note that the greater number of these reports have been issued within the last decade. The earliest work on the variations in milk yield with the size of the cow was by Speir (53, 54) who simply graded the animals as "small, medium and large." He found that the milk and butterfat yields increased with the size of the cows though there was no influence on the butterfat percentage.

The large cows within a breed, according to Woll (67, 68), are as a rule the heaviest producers, while Peters (47) found that the heaviest cows gave the most milk, though it was poorer in butterfat than that of the lighter cows, and Nevens (43) found the yields of milk and butterfat to increase with size. On using 7-day records Gowen (25) found that body weight was closely related to milk yield, while correlations were also found to exist between body length, body width, girth and milk production.

All these sets of data show that there is an increase in production accompanying an increase in the size of the cow, but unfortunately there has been no separation of the animals into age classes, and so there is no possibility of comparing the rate of increase in production with the rate of body growth.

There have been several studies on growth alone in dairy cattle, and of these perhaps the series carried out at the Missouri Station are the most complete. There are two post-natal cycles of growth in the dairy cow according to Brody and Ragsdale (4), while Brody, Ragsdale and Turner (6) state that after 2 years of age the rate of growth declines and the percentage decline in growth with age is constant. The age curves of both linear and tri-dimensional growth, according to Brody (2), may be divided into two segments, one an increasing and the other a decreasing segment, the junction being reached in both cases approximately at puberty.

On grouping records of the various breeds of dairy cattle according to age and weight Brody, Ragsdale and Turner (7) concluded that the increases in fat production and body weight are largely independent of each other though they follow a similar course. It was found by Ragsdale, Turner, Brody, Elting and Gifford (48) that up to 8 years of age milk secretion and body weight in cows on official test increased at the same

rate, while Brody, Ragsdale, Hays and Elting(5) state that the rate of maturity and the weight at maturity are related to duration of life in dairy cattle. The changes in milk flow with age, according to Brody(3), follow the same course as the age changes in body weight, though the milk yield begins to decline some time after the maximum body weight is reached.

From a recent study of body weights and measurements on Ayrshires, Guernseys, Holstein-Friesians and Jerseys, Kay(34) concluded that all breeds reached maturity in skeletal dimensions at about 5 years of age, but continued to gain in weight for a further 2 years, and this period of gain in weight seems to be somewhat more prolonged in the large than in the small breeds. This would indicate that mature body weight is reached at the age generally associated with maximum milk and butterfat yield.

Apparently, therefore, as the cow increases in weight and size generally she increases in milk and butterfat production, but in the light of the evidence at present available it is probably incorrect to say that the increase in production depends entirely, or even directly, on the increase in size or live weight. It is more probable that they are simply concurrent, though perhaps they occur at different rates. It is not possible to be more definite on this point until further evidence on the rate of change with age in the milk and butterfat yields, and especially on the rates of change in body weight and size generally, in the dairy cow are obtained. It is not yet even known with absolute certainty when the dairy cow reaches her maximum body growth.

The increase in milk and fat production, whether or not it is exactly parallel to the increase in body growth, is no doubt due in part at least to the growth of the mammary gland. It is not apposite to go into details here regarding the growth of the mammary gland, but the subject may be reviewed briefly.

The beginnings of the mammary gland are seen in an early stage of the foetus, and there is some growth during intra-uterine development, especially in the later stages. At the birth of the heifer calf the small udder present consists largely of connective, fatty and other tissues distinct from true secretory tissue.

From birth until the age of sexual maturity, or puberty, is reached there is little development in the mammary gland, except perhaps in the non-secretory tissues. At puberty, however, there may be some growth in secretory tissue and there is generally an increase in the fatty and connective tissues.

With the occurrence of each heat period after puberty there is probably associated some growth, not only in the supporting and associated tissues, but also in the true secretory tissue, as it has been shown by Woodman and Hammond (69) that virgin heifers may produce at this time a secretion much like colostrum.

When pregnancy occurs a new period of growth in the mammary gland is instituted. This consists of increases in all the tissues. In the early stages of pregnancy the growth may be hardly noticeable but in the later stages it is very marked. The early growth consists to some extent of both secretory and non-secretory tissue, but as pregnancy advances the growth of true secretory tissue increases and towards the end it is exceedingly rapid. In the latter part of pregnancy colostrum is generally found in the udder, and even in the earlier stages it is sometimes present. According to Hammond (28) little growth of the gland tissue can be observed during the first three months of pregnancy, but at the end of the fifth month the terminations of the secretory ducts swell out and form the alveoli in which the milk is ultimately produced. Up to this time the secretory tissue must have consisted of ducts only. The maximum growth of the secretory tissue of the udder is reached at about the time of pregnancy.

The milk-producing ability of a heifer depends to a considerable extent on the number of active alveoli present in the udder, and with each succeeding pregnancy and lactation there is an increase in the number of alveoli and a consequent increase in milk production. It is not yet known at what age the maximum number of alveoli are found but it is very probable that this occurs about the age of maximum production, and is responsible for it in part at least.

(*b*) *Development.* So far the growth, or formative activity, of the udder, as well as growth in general, has been considered, but attention must also be given to functional activity. It is a well-known fact that practice, or use, gives greater dexterity or facility, and it is very probable that the alveoli, through activity in earlier lactations, become more adept at the production of milk, and so the training of earlier lactations may aid in increasing the production in later years.

Information on the relationship of milk production to growth is scanty, but there is even less available regarding the possibility of a connection between the development of functional activity and milk production. It has been shown by Eckles (11) and Eckles and Swett (14) that bringing heifers into production at an early age is detrimental to their yield, while nothing is to be gained by too great delay. The age at

first calving had an influence upon the size of the animal, though it was found that it was not the bearing of the calf, but the subsequent milk production, which brought about the detrimental effect. As this early milk production checked general body growth it may be assumed that the bad effects on milk production were due in part at least to a checking of the growth of the mammary tissue, though it must be remembered that a great part of this growth of the secretory tissue normally takes place before parturition. In addition the general retarding of the growth of the animal would exert a depressing effect. The decrease in production in this case can be attributed to lack of development in the animal, both in general body activities as a whole and in the secretory tissue of the udder.

A study of Jersey Register of Merit and Guernsey Advanced Registry records, both entry and re-entry, was made by Graves and Fohrman (26). These are the official records of the American breed societies and animals have to meet certain requirements before they are eligible for admission. The cows are generally fitted and forced for these records to a greater extent than would be found in ordinary herd practice, and the first record on which a cow is admitted is classed as her entry record, while subsequent records made by the same cow are called re-entry records. Re-entry records should show an increase over entry records due to the greater age of the animals, but on allowing for this it was found that there was an additional increase in butterfat production of 11 per cent. in the case of the Jerseys and 12·2 per cent. with the Guernseys that could be attributed to the development of the animals through the training they had received in production when they were forced for their earlier records.

On considering cows which had made more than one official record Gaines (17) found that the excess increase obtained, over and above what would be expected from advancing age, was about 8 per cent. of the first record, and he states that the most likely explanation of this excess increase is the artificial factor that there is no commercial object in conducting a second test unless that second test shows a relatively higher production than the first. There is also the possibility of the excess increase being due to a physiological factor in the nature of an increased development of the mammary functions through the exercise and training of the first test. The latter explanation has also been noted by Gowen (23).

Jersey Register of Merit re-entry cows, according to Davidson (9), reach a greater weight at maturity and increase in weight more rapidly

than original entry cows, though both reach their maximum weight at approximately 8 years of age. He assumes that the advantage of the re-entry cows is due to their more favourable care and management. The fat yields of the re-entry cows are greater than the fat yields of the original entry cows, increasing to a maximum at a greater rate with age, and after the peak is reached decreasing less rapidly than the fat yields of the original entry cows. The age at which maximum production is reached is 8 years 9·22 months for the re-entry cows and 7 years 4·42 months for the original entry cows.

The evidence is not as extensive as is desirable, yet it would appear that the increase in production with age is due in part to development, perhaps both of the bodily activities as a whole and of the true secretory tissue in particular. This is most noticeable where animals have been given special opportunities during their earlier lactations as the training in production received then tends to increase their production in later years.

2. *Components of lactation production.*

The ultimate yield of milk and butterfat produced by a cow during a lactation is largely dependent on two components—the initial rate of production, and the persistency with which it is maintained throughout the lactation. Too often in estimating the production of a cow cognisance has been taken only of the initial rate of production, and no attention has been given to persistency. The importance of persistency of production has been shown by M'Candlish, Gillette and Kildee (40). In studying the records of a herd of scrub cows which were being graded up through the use of pure-bred bulls it was found that both initial production and persistency of production improved from generation to generation and resulted in an increased total yield per lactation. Though initial rate of yield and persistency are both important factors in determining the ultimate yearly production of a cow, there has been little attention given to the possible variation of the influence of these two factors with the age of the cow.

(*a*) *Initial production.* In the study of Guernsey and Holstein-Friesian records already mentioned Gaines (17) found that the initial production rate increased with age up to about 7 or 8 years of age, as did the lactation production, while persistency tended to decrease with age. He concluded that the yearly yield is more closely related to the initial yield than to persistency, as, with a constant initial yield, he found persistency to be independent of age. Another conclusion was that

environment has a great effect on persistency, while the influence of heredity on persistency was found to be inconclusive and conflicting. This is opposed to some extent to the results of M'Candlish, Gillette and Kildee (40) already given.

It would appear that the initial rate of production varies with age, probably increasing until the time of maximum yearly production is reached. This is confirmed by the results of short-time tests which were quoted earlier. These were in the majority of cases records obtained early in the lactation and so give some measure of initial production, and they indicate an increase in initial production with age.

(*b*) *Persistency.* It was found by Sanders (50) that persistency varied with age; the older cows tended to be less persistent than the younger animals, while Gaines and Davidson (18) came to the same conclusion, though Gaines (16) states that persistency of production is not affected by age except as age affects the initial rate of yield. In a study of Guernsey yearly records and Holstein-Friesian 7-day records Gaines (17) found that the lactation production increased up to 7 or 8 years of age while persistency tended to decline with age. On working with the records of non-pregnant Guernsey cows Turner (60) found that the average persistency of fat secretion declines rapidly between the second and third lactations as compared to the first, and less rapidly as maturity is reached.

The evidence as a whole goes to show therefore that though initial production increases with age there is a tendency for persistency to decrease with age.

V. SUMMARY.

1. The variations in production due to age have been investigated with the records of 738 Ayrshire cows for 4380 lactations.
2. Milk and butterfat production increase up to about 7 years of age and then show a decrease.
3. The fat percentage for 3-year-olds is higher than that for older cows.
4. After 3 years of age there is little change in the fat percentage with age that is of any practical significance until advanced ages are reached, when there may be a fall of importance.
5. The increase in production associated with age is probably attributable, in part, to the growth of the secretory tissue of the udder and to body growth in general.
6. Part of the increase may also be due to an improvement in functional activity through use.

7. The tendency for milk to show a slightly lower fat percentage as the cow advances in age is probably due to the fact that as the milk yield changes the fat yield changes in the same direction but at a slower rate.

8. There is little known regarding the influence of very advanced age on production, but it is probable that many cows maintain for a long time the production associated with maturity, and then decline slowly.

9. Heifers with a low fat percentage need not as a rule be expected to test higher on reaching maturity.

10. It is probable that the increase in production with maturity is associated more closely with high initial production than with persistency of production.

11. Correction factors for age are presented.

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THE INFLUENCE OF THE NUMBER OF NODULE BACTERIA APPLIED TO THE SEED UPON NODULE FORMATION IN LEGUMES.

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(With One Text-figure.)

It is natural to suppose that the greater the number of nodule bacteria in the soil surrounding their host legume, the better will be the chances of any given root hair becoming infected. The number of nodules formed should, in consequence, increase as the number of these bacteria, unless other factors limit the infection or subsequent nodule development.

This problem of the factors limiting infection has a bearing upon a related but less simple problem which is of importance to the practice of legume seed inoculation, namely: how far can the number of bacteria added to each seed be increased with a corresponding improvement in the number of nodules formed?

This question was investigated by A. T. Perkins (1), who made experiments with Soybeans grown in sand cultures, and inoculated before sowing with varying numbers of nodule bacteria. He found that the number of nodules was increased when the number of bacteria per seed was raised from 1 to about 50, but that more bacteria than this did not produce more nodules. From this result he concludes that "after a certain degree of infection is reached the host is immune to further infection." The danger in drawing such a conclusion lies in the implied assumption that the number of bacteria added to the seed is similar, or at any rate proportional, to the number subsequently surrounding the root hairs. When inoculated seed is sown in soil or sterile sand the bacteria spread into the surrounding medium and increase up to a limit presumably set by the food supply or by competition with other organisms. This spreading and multiplication has been studied in sterilised soil by Thornton and Gangulee (2). Perkins used sand containing nutrient salts but lacking in organic energy material which was presumably limited to substances produced from the plant's roots by secretion or by the decomposition of dead rootlets. Only 1 lb. of sand was used per

pot containing 10 plants. Under these conditions it seems probable that the multiplication of the bacteria would soon be arrested by lack of energy material even when a small initial number of cells were introduced, so that a heavier inoculation would not produce a larger population surrounding the roots. In soil under field conditions, on the other hand, the plant's roots penetrate a larger volume of a substratum comparatively rich in energy material. The bacteria introduced on the inoculated seed will consequently be able to multiply to a much greater extent before their numbers are limited by lack of food material and until this limit is reached the number of cells originally added should affect the size of the population in the soil. Under field conditions, therefore, it was to be expected that the number of bacteria introduced on the seed should have an influence on the population of the bacteria surrounding the plant's roots even when large numbers were compared. Whether this difference in population would produce a difference in nodule numbers should be determined by the point at which the plant was able to resist further infection. There are thus two factors, either of which may set a limit to the effectiveness of increasing the dose of inoculum upon the seed, first, the maximum population of infecting bacteria attainable in the neighbourhood of the roots and secondly, the resistance to infection of the plant.

In actual farming practice another factor is introduced by the fact that the farmer is often unable to sow his seed immediately after inoculation. The consequent storage of the seed may therefore allow time for the death of many of the bacteria to take place. To approximate to practical conditions, therefore, the effect of varying concentrations of bacteria upon the seed must be tested both when the latter is sown at once and when it is stored for varying periods after inoculation and before sowing.

In the following field experiment the seed was inoculated at rates of one culture to 7, 14, 28 and 56 lb. of seed and, in order to test the power of the organisms to remain viable on the seed, each dose of inoculation was tried after storing the seed at room temperature for 1, 7, 14 and 28 days. The cultures used for inoculation were grown upon an agar medium containing extract of lucerne roots, mineral salts and saccharose, and the inoculation was carried out by means of a suspension of the bacteria in skim milk containing 0·1 per cent. of $CaH_4(PO_4)_2 + 2H_2O$ (Thornton and Gangulee (2)). In making each inoculation, a suspension of the bacteria from four cultures was made and suitably diluted.

The trial was carried out on Colonel E. P. Brassey's estate at Upper Slaughter, Gloucestershire, the farming operations being supervised by Messrs C. Comely, of the County Education Office, and Mr W. H. Blake. The soil is shallow and calcareous, overlying flaggy limestone. It had been shown in a previous experiment on an adjacent field that uninoculated lucerne plants were practically free from nodules. The land used for the experiment had previously carried winter barley, folded off to sheep and had received no nitrogen other than that supplied by their droppings. The ground was on a slight slope and there was some gradient in fertility. To reduce the effects of this, each treatment was tested on duplicate plots and three were sown with uninoculated seed, the plots having the random arrangement shown in Table I, but with the two blocks placed in a single row. The total area of the 35 plots was 1⅞ acres. The seed was sown with hand drills on July 21 to 23, 1927, at the rate of about 18 lb. to the acre. The flow of seed through the drill was somewhat uneven. This seems to have influenced the subsequent growth.

Table I.

Block A

Pot No.	2	4	0	5	7	13	15	16	9	12	0	6	8	14	11	3	1	10
Lb. of seed treated per culture	56	56	Un-treated	28	28	7	7	7	14	14	Un-treated	28	28	7	14	56	56	14
No. of days the inoculated seed was stored	14	1	,,	28	7	28	7	1	28	1	,,	14	1	14	7	7	28	14

Block B

Pot No.	1	16	13	4	15	9	8	3	5	6	14	10	0	7	2	11	12
Lb. of seed treated per culture	56	7	7	56	7	14	28	56	28	28	7	14	Un-treated	28	56	14	14
No. of days the inoculated seed was stored	28	1	28	1	7	28	1	7	28	14	14	14	,,	7	14	7	1

The plant germinated well but during August it was attacked by millipedes and beetles and suffered a good deal of temporary damage. On September 24 sample plants were dug up, about 30 plants being taken from each of four different areas on each plot. These were taken to the laboratory, their roots washed out and the nodules on 50 plants from each plot were counted. The results of this count are shown in Table II. Increase in the strength of the inoculum has increased the nodule numbers up to the strongest dose used. But if the nodule numbers are plotted against the quantity of culture per lb. of seed, the curve rises steeply from uninoculated to the weakest inoculum tested and is flatter over the range covered by the experiment. The effect of increasing

doses is thus less over this range than it would be at a weak concentration (Fig. 1).

The effect of storing the inoculated seed shows that the bacteria are remarkably resistant to drying on the seed. Seed stored for 14 days

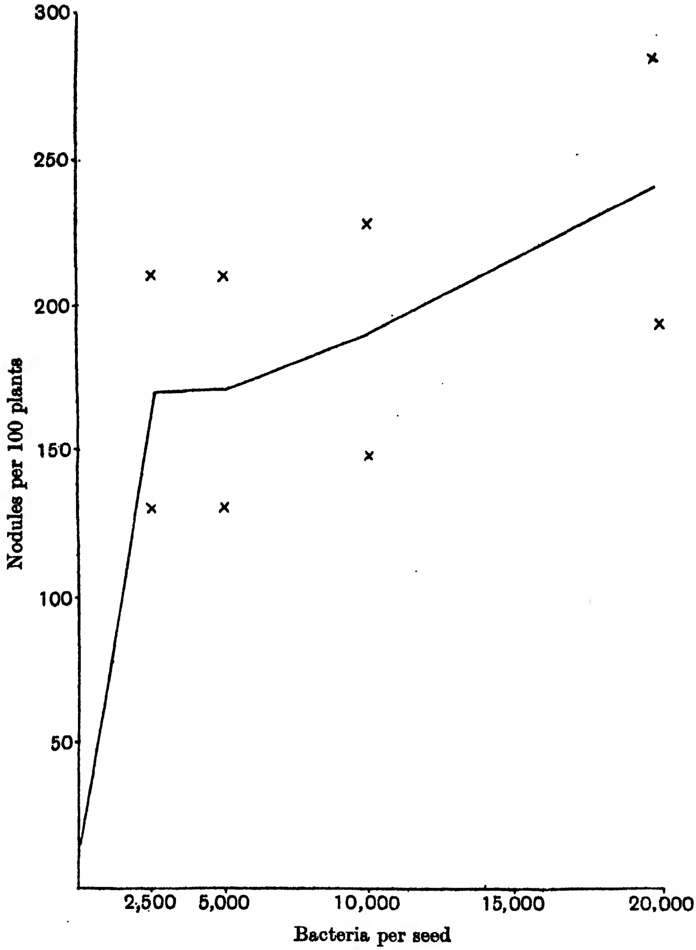

Fig. 1. Relation of nodule numbers to bacteria per seed.
The crosses represent the range covered by 3 times the standard error.

after inoculation still produced 61 per cent. of the number of nodules obtained from seed stored only 1 day. The effect of storage is greatest between 1 and 7 days. It seems therefore that the number of bacteria surviving upon the seed falls off most rapidly during the first few days.

The case may thus be compared with that of a suspension of bacteria or fungus spores in the presence of a killing agent (Henderson Smith (3)).

Table II. *Nodule Counts, Slaughter Field Trial, September* 1927.

Comparison of Parallel Plots.

Period of storage (days)	1 culture to 7 lb. seed: Total nodules on 50 plants	X^2 of duplicate plots	1 culture to 14 lb. seed: Total nodules on 50 plants	X^2 of duplicate plots	1 culture to 28 lb. seed: Total nodules on 50 plants	X^2 of duplicate plots	1 culture to 56 lb. seed: Total nodules on 50 plants	X^2 of duplicate plots
1	165, 176	0·355	154, 130	2·028	188, 119	15·508	79, 124	9·975
7	102, 111	0·380	66, 82	1·730	53, 49	0·157	112, 117	0·109
14	86, 121	5·918	100, 99	0·005	67, 73	0·257	67, 79	0·986
28	98, 96	0·021	76, 50	5·365	97, 36	27·977	47, 55	0·627
Total X^2		6·674		9·128		43·899		11·697

(Total X^2 expectation 4·00.)

Comparison of Treatments.

Period of storage (days)	Total nodules on 100 plants and standard error 7 lb. seed	Total nodules on 100 plants and standard error 14 lb. seed	Total nodules on 100 plants and standard error 28 lb. seed	Total nodules on 100 plants and standard error 56 lb. seed	Means	Percentage of numbers obtained with fresh inoculum
1	341 ± 37	284 ± 34	307 ± 35	203 ± 28	283·75	—
7	213 ± 29	148 ± 24	102 ± 20	229 ± 30	175·25	61·75
14	207 ± 29	199 ± 28	140 ± 23	146 ± 24	173·0	61·00
28	194 ± 28	126 ± 22	133 ± 23	102 ± 20	138·75	48·9
Means	$238{\cdot}75 \pm 15{\cdot}4$	$189{\cdot}25 \pm 13{\cdot}8$	$170{\cdot}5 \pm 13{\cdot}1$	$170 \pm 13{\cdot}0$		
Percentage of numbers obtained with strong inoculum		78·5	71·4	71·2		

Notes. The standard errors are based on the sum of the X^2 indices of the 16 sets of duplicates, and are actually calculated by taking twice the square root of each total count (cf. method developed by Fisher, Thornton and MacKenzie (4)) (3 times the standard error is a significant difference). 50 plants from each of the uninoculated plots bore 12, 6 and 4 nodules respectively.

In order to test whether the differences in nodule numbers, produced by varying concentration of culture and time of seed storage, were sufficient to affect the crop, weighings of the latter were taken. Table III shows the yield in lb. of green lucerne from 0·013 acre from each plot. The plots were very uneven as can be seen by comparing parallel plots. This seems to have been due to two causes. In the first place, the lower part of the field, bearing plots 9, 8, 3, 5, 6, 14, 10, 0, 7, 2, 11, 12 in Block B (Table I), had a much higher fertility than the remainder of the area. In the second place, uneven running of seed from the drills may have caused some plots to receive rather too little seed. The results of the weighings show, however, that there are no significant differences due either to amounts of culture or to length of time of storage in spite

of the fact that inoculation has, on an average, increased the yield over untreated by about 100 per cent. The differences in nodule numbers found on the 2 months old inoculated plants have not been sufficient to affect the yield perceptibly.

Table III. *Upper Slaughter Lucerne Experiment.*

Result of weights taken from duplicate plots, September 14, 1928.

Period of storage (days)	Lb. of seed per culture: 7	14	28	56	Means lb.
1	2000 1769	2769 1461	2538 1769	1538 1846	1961·25
7	1307 1307	2884 1538	2461 1615	2846 2000	1994·75
14	2654 2000	2346 1807	2692 1807	2961 2499	2345·75
28	1538 1307	2807 1692	2807 1923	1615 1923	1951·5
Means	1735·25	2163·0	2201·5	2153·5	2063·3

Untreated plots: 961, 691, 1384. Mean of untreated plots: 1012.

In the experiment above described, large numbers of bacteria were added per seed. A culture contains on an average 28,000,000,000 viable organisms. A pound of lucerne seed contains about 200,000 seeds, so that the number of organisms added per seed in the experiment ranged from 2,500 to 20,000. At these heavy concentrations a maximum effective dose was not reached under field conditions. In order to see whether a maximum effective inoculation could be reached by still heavier doses of the organisms, a pot experiment with Runner Beans (*Phaseolus multiflorus*) was made. In order to lessen the likelihood of the soil becoming saturated with the bacteria and thus affording a limiting factor, large pots were used containing 25 kg. of a mixture of equal parts Rothamsted unmanured soil and sand. This was maintained at a moisture content of 14·0 per cent., loss of water being determined by weighing each time the pots were watered. The following treatments were tested:

A. Uninoculated.
B. Seed inoculated at the rate of 1 culture to 16 lb. of seed.
C. Seed inoculated at the rate of 1 culture to 4 lb. of seed.
D. Seed inoculated at the rate of 1 culture to 0·5 lb. of seed.
E. Seed inoculated at the rate of 1 culture to 0·5 lb. of seed and 4 oz. of chaff mixed into the soil + sand.
F. Plants repeatedly watered with a thick suspension of the bacteria.
G. Plants repeatedly watered with a thick suspension of the bacteria and half of their leaves removed.

The cultures used contained on an average 40,000,000,000 viable organisms, and the numbers added per pot on the inoculated seed were approximately 40,000,000 in series B, 160,000,000 in series C and 1,280,000,000 in series D. In series E, seed treated with the heaviest of these doses was sown in soil to which chaff had been added, it being known that the number of nodule organisms are thereby increased. In series F and G repeated doses of inoculum were added during the growth of the plants by pouring thick suspensions of the bacteria onto the soil[1]. In this manner about 150,000,000,000 organisms were added to each pot.

The numbers of nodules that developed in each series are shown in Table IV, in which the numbers per gram of root are calculated as this gives a better measure of the infection where the mass of the roots is variable.

Table IV. *Pot Experiment with* Phaseolus.

Nodules per gram of root.

	Block No.						
Series	1	2	3	4	5	6	Mean
A	83	81	52	108	69	55	78
B	84	64	98	130	114	111	100
C	95	64	90	53	100	50	75·3
D	94	164	119	95	136	218	137·3
E	165	156	224	315	91	116	178
F	234	329	284	284	288	—	284
G	215	390	219	·450	199	154	271

The soil was not sterilised, since the establishment of the introduced organisms amongst the existing soil population was one of the factors to be considered. It contained a natural population of *Phaseolus* nodule bacteria, as is shown by the development of nodules on the uninoculated plants. The addition of 160,000,000 bacteria to the seed in series C has not increased the number of nodules but there is an increase produced by adding 1,280,000,000 in series D. The heavy dose needed to increase the nodules over control perhaps indicates a large population of *Phaseolus* bacteria in this soil. The increase is rather larger where chaff has been incorporated into the soil. In series F and G the nodules numbers have

[1] Six parallel pots were set up in each series, six seeds being sown per pot and the seedlings subsequently thinned out to three plants per pot. In series B, C and D the seed was inoculated by wetting with a suspension of bacteria in skim milk containing 0·1 per cent. $CaH_4(PO_4)_2 + 2H_2O$. The thickest suspension used was of such a strength that the milk just absorbed by $\frac{1}{4}$ lb. of seed contained the bacteria from one culture. Weaker suspensions were obtained from this by dilution. The pots were arranged in the glasshouse in six blocks each containing one pot of each treatment. Within each block the pots were interchanged in position at intervals. The seeds were sown on August 15, 1927, and the experiment terminated on October 15. The roots were washed, the nodules counted and the dry weights of tops and roots obtained.

been approximately doubled as compared with the heaviest seed inoculation in series D. In series G the removal of half the leaves caused a reduction in mean root weight to 1·45 gm. as compared with series F where the mean weight was 3·94 gm., but the number of nodules per gm. of root was not changed by the defoliation.

In this experiment, as in the field trial, no maximum effective dose of inoculum could be found. A comparison of this result with that obtained by Perkins (1) confirms the view that the effectiveness of his higher doses of inoculum was limited by the lack of sufficient food material in the sand to support any but a very small population of nodule bacteria. On the other hand, although the maximum effective dose was not passed in the experiments here discussed, the increases in nodule numbers have not been at all in proportion to the increased doses. Thus series D received about 1,280,000,000 organisms per pot and series F about 150,000,000,000, but this enormously increased dose has only doubled the number of nodules produced. The small effect of large doses of bacteria in increasing nodule numbers may be due to the population of nodule bacteria surrounding the roots being but little affected by the number originally added to the soil. Or it may result from a true immunity of the plant to heavy infections. The examination of root hairs of legume plants growing on agar seems to show that the plant cannot be infected by the organism to an indefinite extent. The following observations were made on young lucerne plants grown in wide tubes on agar under aseptic conditions and inoculated with a pure culture of the nodule organism. The absence of other bacteria from the tubes was checked by plating at the conclusion of the experiment. When the plants were about 6 weeks old the roots were examined and it was found that large numbers of the bacteria were clustered round the root hairs and that motile forms were also present. Nearly every root hair had a number of bacteria surrounding it. It was easy to see the infection threads entering the root hairs. In each of six plant roots, 100 root hairs, taken at random, were examined and the number found to contain infection threads in the six samples were 6, 3, 2, 8, 2 and 3. It would appear therefore that only a few of the root hairs actually become infected even where large numbers of the organisms are present amongst them. There would appear to be some factor other than the mere presence of the organisms that controls their entry into the root hairs.

SUMMARY AND ABSTRACT.

In a field trial with lucerne grown from seed treated with varying doses of culture it was found that the numbers of nodules were increased as the dose was raised from 2,500 to 20,000 organisms per seed (56 to 7 lb. of seed per culture). Storing the seed for periods up to 28 days between inoculation and sowing, caused some loss in the nodule numbers. This loss was greatest between 1 and 7 days' storage.

The difference in dose of culture and in period of storage did not significantly affect the crop subsequently obtained from the inoculated plots, whose yield was, however, much above the uninoculated.

In a pot experiment made with runner beans, it was found that increase in the dose of culture above 1,280,000,000 organisms per pot containing six seeds was still capable of increasing nodule numbers but not to an extent proportional to the increase in dose.

The experiment does not exclude the possibility that the restriction in effect of very heavy doses may be due to the soil population becoming saturated with the bacteria. On the other hand, observations on lucerne plants grown aseptically on agar and inoculated with a pure culture, showed that even when excessive numbers of the bacteria immediately surrounded the root hairs, only 4 per cent. of these were infected.

ACKNOWLEDGMENT.

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VARIATION IN THE DURATION OF GESTATION IN THE GOAT.

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(With Two Text-figures.)

CONTENTS.

Introduction.

The subject of the duration of gestation in man is one which has been well explored and many facts of interest elucidated from the available data. Although, however, much may be deduced, the results obtained are open to a certain amount of question owing to the fact that fertile coitus may occur at any time in the menstrual cycle (Asdell (1)). This introduces other factors modifying the duration of gestation which may or may not be constant for the problems investigated. Amongst these problems have been the effect of the time of the year during which the pregnancy began, the effect of the degree of parity, and of the number of offspring at a birth.

For all these questions, the answers to which raise questions of considerable physiological interest, much more reliable information may be obtained from the domestic animals. This is for two main reasons, first, that the date of fertile coitus may be given with much greater accuracy, avoiding the possibility that any one of a number of unions spread over a considerable period in a single menstrual cycle may have been the fertile one. In man, because of this possibility, the inception of gestation is usually referred to the date of the appearance or of the cessation of the

last menses. The second reason is that in the domestic animals, usually with a short season in which coitus may occur, the amount of variation in the length of the interval between the time of insemination and of fertilisation is lessened. Analysis of the data presented in this paper has shown this to be an important factor.

In a survey of the literature for facts which would serve as the basis for exploring the physiological laws governing the length of pregnancy, we were impressed by the lack of information on the subject in the domestic animals, the most valuable material we could have. The reports are few and far between, while those which are presented in a form in which they can be used are very scanty indeed. By far the most valuable paper was that of Sabatini (2) who showed that considerable breed differences occur in the duration of gestation in the sheep, pig, horse and cattle. He also gives extensive data to show that the fact that the male foetus is borne longer than the female is true for other species besides man. He is one of the few authors who have given the frequency distributions so that the significance of his results may be checked by the use of statistical methods. So far as we can ascertain, Berry Hart (3) alone has treated the problem statistically. He took Earl Spencer's (4) figures for cattle, Tessier's (5) for cattle and sheep, and Reid's (6) for man and showed that the frequency curves which may be drawn from these data are all symmetrical curves not differing widely from one another. It is evident from Hart's curves that the length of gestation groups itself around a single mode and consequently the factor or factors inducing parturition act with greatest intensity at a definite interval after conception. They give no indication of the cause of the variation which is observed.

The possession by the writer of information regarding a large series of gestations in the goat has enabled a more careful survey of the variations which occur to be made than has previously been the case. In order to correlate the results obtained with those given by other animals, material already existing in the literature has been drawn upon and the constants for each set calculated.

During the course of the analysis of the goat records, a correction was applied, which, by removing the extremes in duration, lessened the spread and hence the variation. A proportionate correction has been applied where necessary to the borrowed data in order that they may be more strictly comparable with our goat data.

Duration of gestation in the goat.

The data on which these figures are based are taken from the Kid Register of the British Goat Society. This register exists as a series of entry forms filled in by the owners of the goats. It is, therefore, free from errors of transcribing and of printing, an important factor which is worth the attention of those who rely on Herd Book data for their source of information. It was found on enquiry amongst the breeders

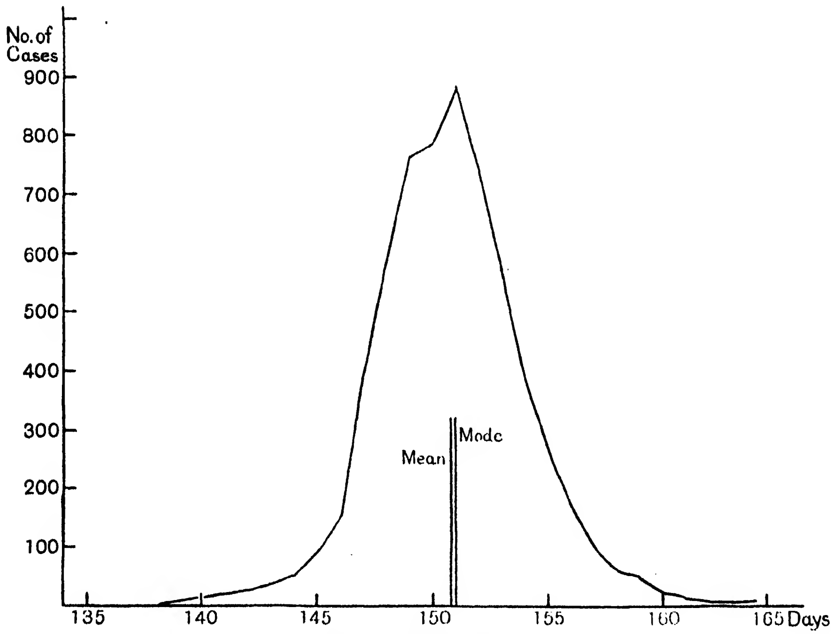

Fig. 1. Frequency curve of duration of gestation in goats.

concerned that certain entries concerning the possession of horns by the goats were accurate in the manuscript Kid Register and erroneous in the printed Herd Book. Amongst the data recorded is the date of service of the dam and of the birth of the kid. From these the length of gestation was calculated. In those few cases where two consecutive days were given for the service, the earlier was taken as the fertilising service. If more than two days were given, or if there was an interval between the days, the case was omitted. Usually the goat is served towards the beginning of the heat period, which may last for three days. Precautions were taken against counting the same gestation more than

once, *i.e.* in the frequent cases in which twins or triplets, etc., were registered. The records were spread over 50 years ending in 1923. The possibility of variation due to climatic conditions which change from year to year was thus eliminated to an extent. From subsequent work it seems that this may be a factor of some importance. The goats included pure Anglo-Nubian, Toggenberg, a few Saanen and English together with a few of other breeds of milk goats, but the great majority were a mixed breed described as Anglo-Nubian-Swiss, but more recently designated "British." In many cases gestations of the same dam over a series of years could be compared. There seems to be considerable individuality displayed by the dams in this respect.

The total number of separate gestations recorded is 6342 with a duration varying from 107 days to 200 days. The mode is at 151 days with 891 cases. The curve is almost symmetrical and falls off rapidly, the daily number of cases becoming less than 100 at 6 days from the mode.

In the outlying regions of the curve a very small hump of 10 cases rising from 1 and 3 occurs at 137 days, and another of 7 at 119 days. The first hump at 137 days occurs 14 days from the mode, or approximately the difference between the end of one heat and the beginning of another. This may represent a real physiological inclination towards birth at a heat-period interval, or it may represent a few cases in which oestrus and service have occurred during the early part of gestation. In connection with the possibility that the rise denotes the occurrence of premature birth, it must be pointed out that the rules of entry to the Kid Register required that application for the entry should not be made until the kid was one month old. Special precautions are not likely to be taken to keep alive the premature kids of the goat, a very fecund and relatively inexpensive animal, although the possibility arises, as many of the goats listed ranked as household pets. The other maximum at 119 days is 30 days from the mode and apparently represents those cases in which a mistake in the month has been made on entering. Probably some of the other entries at the extremes, which spread rather widely, are due to the same cause.

On the upper side of the mode there is very little rise at the next oestrous cycle interval or at the month interval, but in both cases the possibility of error must be borne in mind, *e.g.* the occurrence of an additional service without record or of a mistake in the month.

One has hesitation in accepting these extreme cases for non-physiological reasons, and after carefully considering the figures it was felt that

a justifiable precaution would be to eliminate the ends of the curve. Accordingly only those cases between 138 and 164 days inclusive were kept and the remaining 57 cases under and 59 above were discarded. This represented a total elimination of 1·83 per cent. of the whole series.

The mean of these 6226 cases is 150 ± 0·003. The average period of gestation for the mixed type of goat found in Great Britain is therefore approximately 151 days.

Table I.

Length of gestation (days)	Frequency	Length of gestation (days)	Frequency	Length of gestation (days)	Frequency	Length of gestation (days)	Frequency
107	*1*	*135*	*2*	154	392	*173*	*3*
112	*1*	*136*	*1*	155	262	*174*	*3*
116	*1*	*137*	*10*	156	167	*175*	*4*
117	*2*	138	3	157	97	*176*	*1*
118	*2*	139	9	158	59	*177*	*1*
119	*7*	140	14	159	52	*178*	*2*
120	*2*	141	16	160	28	*179*	*5*
121	*2*	142	18	161	17	*180*	*2*
122	*2*	143	37	162	12	*181*	*3*
123	*4*	144	50	163	6	*182*	*3*
124	*3*	145	92	164	9	*183*	*2*
126	*1*	146	148	*165*	*6*	*184*	*1*
127	*2*	147	394	*166*	*4*	*185*	*1*
129	*1*	148	585	*167*	*3*	*186*	*1*
130	*3*	149	759	*168*	*1*	*187*	*1*
130	*3*	150	787	*169*	*3*	*190*	*1*
132	*1*	**151**	**891**	*170*	*2*	*200*	*1*
133	*3*	152	739	*171*	*1*		
134	*3*	153	583	*172*	*4*		

The standard deviation of the curve is 3·26 and the coefficient of variation is 2·16. This is very low for biological material even though the narrowing down of the data has reduced the C.V. in this case. It may be taken as indicating that there is a very definite and constant mechanism by which the date of parturition is governed.

But little stress can be put on this mean figure for the duration of gestation for it represents a series of gestations from goats of many types. By far the greatest number were Anglo-Nubian-Swiss type, a predominatingly Swiss type, there being little of the English or Nubian in its appearance. That there is some breed difference is evident, for a smaller series of goats, independent of the major series treated above, largely Anglo-Nubian with a few other types intermixed, gave an average gestation of 150·0 ± 0·1 days (279 cases). The difference is small, and, as pure-bred goats in the sense of descent from a single type are very scarce, the difference hardly merits further pursuit with a view to more accurate study. It is well known that similar differences occur in other species, for instance, the duration of gestation in Shropshire

sheep is 145·61 ± 0·08 days, while that of wool Merinos is 151·22 ± 0·07 days (data calculated from figures given by Sabatini). The difference cannot be one of size, for, in horses, saddle horses have a relatively long gestation period, *i.e.* 345·4 ± 0·3 days for pure-bred Kladruber, and for Rhein-Belgian draught horses the average is 332 ± 0·2 days, while the Percheron has a gestation of 342·2 ± 0·4 days, more nearly approaching that of the saddle horse. A similar circumstance is seen in two strains of beef shorthorns. The Durham shorthorn has an average gestation of 284·7 ± 0·1 days, while the Booth shorthorn has one of 280·9 ± 0·1 days. Here the difference cannot be explained away either by differences of species or of sub-species but is evidently a family trait, possibly a difference of management, though that hypothesis is doubtful.

The very slight degree of asymmetry found in the frequency curve—with the suspicion of a second mode between 149 and 150 days—is possibly due to the inclusion in the data of this Anglo-Nubian type with its somewhat lower gestation period. The tendency towards bimodality is very slight, and if allowance be made for this disturbing factor the distribution may be regarded as quite symmetrical. The mean and mode differ by 0·2. Comparing the average gestation period of 150·807 ± 0·003 obtained by us with figures quoted for the goat by others, we have:

	Days
Richards (7)...	150
Richards (7) (for large Swiss goats)	152–154
Mayall (8)	154
Holmes-Pegler (10)...	147–152
Davies (11)	150
Crepin (9)	154

THE DURATION OF GESTATION ACCORDING TO THE MONTH OF CONCEPTION.

In considering this aspect of the duration of gestation the limits of variation were taken at 139 and 163 days inclusive. The gestations were arranged according to the month in which conception occurred. October was the most frequent month with May the least frequent.

The average length of gestation was highest with August conceptions at 151·3 ± 0·1 days and least with June conceptions at 149·7 ± 0·4 days. The latter appears to be an anomalous average for it is the only average of the twelve months which does not fit the otherwise regular curve of monthly durations. It occurs where the number of cases is small and therefore shows a high probable error. The true minimum seems to be the February conceptions at an average duration of 149·8 ± 0·1 days.

The difference between the maximum and minimum is 1·5 days. This is certainly a significant difference for the probable error of each is but ± 0·1 day. The differences from month to month are not always significant, but except for the June average are regularly placed on a continuous curve. It is evident, therefore, that there is an actual difference in the length of gestation in the goat according to the time of year at which service takes place.

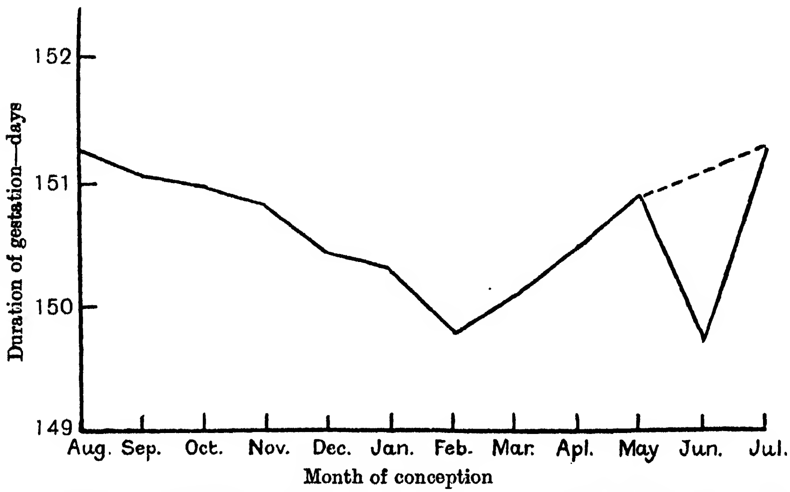

Fig. 2. Relation between duration of gestation and month of conception in goats.

A possibility is that the average length of gestation, if measured from the time of actual fertilisation of the ovum to parturition, may be constant. This hypothesis would imply that there is a difference in the length of the interval between coitus and fertilisation of 1·5 days from the one month to the other. The duration of oestrus in the goat is 2 to 3 days but nothing is known of its variation at different times of the year, or of the relation of the time of ovulation to the period of oestrus in the goat. No definite conclusions can therefore be drawn regarding this hypothesis.

Another possibility is that the greater length of a gestation following service in the autumn months is due to a curtailment in the nutrition for the foetus, either directly due to a reduction in the amount available for the month, or indirectly, from the greater amount of the energy intake used to maintain the body temperature. In the February conceptions, after which gestation is shortest, either factor may have an influence, as the foetus is carried during a time of increasing air temperature and increasing food supply. This difference of 1·5 days is small in

a gestation of 151 days, and its smallness may be due to the fact that the recorded goats, being kept as individuals or in small herds by owners who are not specially anxious to make a profit, are exceptionally well fed and tended during the winter months. The food shortage to the dam is accordingly small and cannot be stressed as of great importance. It is probable that the variation is due to a combination of both circumstances, interval between insemination and fertilisation and also energy supply. It is important to note that the procession of the average length from month to month throughout the year is continuous.

Wellman (12), working on a series of 5437 horse records, finds a procession in the average length of gestation also, but it is discontinuous. In July parturitions the average is 321·3 days, it then rises continuously to the May parturitions when the gestation lasts for 346·1 days, a difference of 24·8 days, a very high and somewhat astounding figure. It is curious that no parturition occurred in the intervening month of June, although in May there were 163 and in July 172 births. One cannot invoke changes in the nutrition of the embryo as an explanation of this difference as the period of gestation is 11 months, and there is but an interval of a month between these extremes. Wellmann regards the difference as due to some change in the properties of the sperm.

Wellman also gives monthly series for cattle of Simmentahl and Hungarian breeds. The month of parturition which coincides with long gestation in the Simmentahl breed agrees exactly with the month showing a short average in the Hungarian breed. Wellman in no case gives distributions, so that the probable errors of his averages cannot be worked out. The differences in the averages are 17·1 days and 7·6 days respectively.

In man, according to Issmer (13), births which occur during the months October to March have an average gestation period of 279·5 days, while in those occurring from April to September the period is 277·2 days, a difference which is more in proportion to that in the goat, but, as the period with long gestations gives a conception period corresponding to the spring and summer, variation tends to be in the opposite sense to that in the goat. The period of gestation in man, however, occupies too long a period of the year for just comparison with that of the goat.

As they stand these results tend to confuse the issue and fail to provide any clue to the nature of the factor or factors which produce this progressive alteration in the duration of gestation in the goat.

It is noteworthy that the variability of the duration of gestation is least when the duration of gestation is least. This suggests that

whatever the factor causing the lengthening of gestation may be it is not at all constant in its action. The shorter gestation must be taken as that which is less complicated by disturbing factors, and should be regarded as the true duration of gestation for the species.

Table II. *The duration of gestation according to the month of conception—goats.*

Month of conception	Duration of gestation (days)	No. of cases	Standard deviation	Coefficient of variation
August	151·3 ±0·1	311	3·21	2·12
September	151·07 ±0·07	917	3·23	2·14
October	150·97 ±0·05	1793	3·11	2·06
November	150·85 ±0·05	1495	3·15	2·09
December	150·42 ±0·07	757	2·92	1·99
January	150·3 ±0·1	399	3·21	2·13
February	149·8 ±0·1	219	2·84	1·89
March	150·1 ±0·2	168	3·51	2·34
April	150·5 ±0·4	61	4·37	2·90
May	150·9 ±0·5	27	4·06	2·69
June	149·7 ±0·4	43	3·79	2·53
July	151·3 ±0·2	86	3·35	2·21

The influence of the age of the dam.

When the dam was entered in the Kid Register or in the Herd Book it was possible to secure a record of her age at the time that the kid registered was born. The duration of gestation figures could not be arranged according to the numerical sequence of the gestations as it was never certain that all the gestations were represented, especially in the case of the first, for custom varies in initially serving the doe in her first or second year. Most breeders, however, agree in delaying this service until the goat experiences its second series of oestrous cycles.

The dams were arranged in classes of one year intervals making the first class 0 y. 3 m. to 1 y. 3 m. The month of greatest frequency of births is March and that of greatest frequency of conceptions is October. Hence this method of division causes the times of oestrus and conception of practically all the goats born as the result of any one breeding season to fall within the class, the greatest frequency being about the middle of this class. There is very little overlapping with this method of division, the class year beginning with the lowest ebb of reproductive efficiency. Occasionally a goat produces two sets of kids in one year. When this occurs both gestations are, of course, credited to the same year. There are 20 such cases in a total of 3898 gestations. In many cases a long series of gestations of a single dam is recorded. The greatest age at which a parturition occurred was 16 years.

In calculating the averages and constants, gestations below 138 days and above 164 days have been omitted for reasons already given.

The average gestation for 0 y. 3 m. to 1 y. 3 m. is 150·1 ± 0·1 days, while that for 1 y. 3 m. to 2 y. 3 m. is 150·61 ± 0·06 days, a significant difference. As a great many, probably three-fourths, of the 1 y. 3 m.–2 y. 3 m. goats are kidding for the first time and the 2 y. 3 m.–3 y. 3 m. class with probably few first kidders shows but a small rise, the difference in the first two classes must, if no other factor is influencing the length of pregnancy, be related to the age of the goat and not to the parity. There is a small but continuous rise in the length of gestation from the 1 y. 3 m.–2 y. 3 m. class to the 5 y. 3 m.–6 y. 3 m. class. This change taken in the aggregate is significant. Above this age the changes are irregular and as the number of cases is becoming low the probable error increases considerably.

Table III. *Relation between age of dam and length of gestation—goats.*

Age at conception (y. and m.)	Gestation average (days)	Standard deviation	Coefficient of variation	No. of cases
0. 3–1. 3	150·1 ±0·1	3·30	2·20	307
1. 3–2. 3	150·61 ±0·06	3·29	2·19	1193
2. 3–3. 3	150·80 ±0·08	3·26	2·16	819
3. 3–4. 3	150·84 ±0·09	3·27	2·17	554
4. 3–5. 3	150·9 ±0·1	3·11	2·06	390
5. 3–6. 3	151·3 ±0·1	3·34	2·21	263
6. 3–7. 3	151·2 ±0·2	3·27	2·16	167
7. 3–8. 3	150·9 ±0·2	3·19	2·11	108
8. 3–9. 3	151·1 ±0·2	2·30	1·52	53
above 9. 3	150·6 ±0·4	3·45	2·29	44

Further evidence that the length of gestation is related to the age of the dam rather than the size of the litter (which in most species is lower with a young parent) is adduced from the data described below. In that work the degree of parity was found to have little, if any, influence, the trend being towards a decrease in the length of gestation with increasing degree of parity. There is, then, some influence at work in the immature animal which decreases the length of gestation to a slight extent. In the young growing animal one would expect a smaller amount of nutrition to be available for the foetus, hence a longer gestation, but this is not the case. Is the ratio, size of foetus to size of dam, an influencing factor?

The size of the litter.

For work on the influence of the size of litter on the duration of gestation, the large series used so far was of no avail, as the Kid Register

consists of entries of individual kids. A small number of accurate records, 279 in all, was obtained from three sources:

(1) Records of births in a few herds entered in the year book of the English Goat Society (now extinct). Occasionally, one or more of the kids were recorded in the Kid Register from which the duration of gestation could then be obtained.

(2) Private records of Mrs C. Pickard.

(3) Private records of the late Mr B. Ravenscroft.

The goats were for the most part Anglo-Nubian with a few Swiss. The average duration of gestation was 150·0 ± 0·1 days, 0·8 day below that of the larger series already discussed. The difference in breed composition of the two samples accounts for the discrepancy.

The number of cases for each litter size is small and the probable error correspondingly large. Accordingly there is no reliable indication of a difference in the duration of pregnancy with a change in the number of developing embryos, though what variation there is points to a reduction in the time as the size of the litter increases.

Table IV. *Size of litter and length of gestation.*

Numbers in litter	Length of gestation (days)	No. of cases
1	150·1 ± 0·3	58
2	150·2 ± 0·2	150
3	149·7 ± 0·2	63
4	148·9 ± 0·5	8

COMPARISON OF THE GOAT CURVE WITH THOSE OF OTHER SPECIES.

In order to compare the results from the goat records with those from other animals, the literature was searched for reliable data which could be worked up with this object in view. To be used for this purpose the data had to give the type of animal, and the frequencies at suitable class intervals, one day being the usual interval. The mean together with its error, the standard deviation and the coefficient of variation could then be calculated for each set. In doing this it was obvious that as the extremes of the goat curve had been eliminated other data must be similarly treated, or the goat data, being more homogeneous, would show comparatively little variation. As the effect of the goat correction was to remove the cases in which the month had been mistaken, *i.e.* where the name of the month had been wrongly entered, a possibility was to take exactly the same number of days on either side of the mean for other animals as for the goat. However, if the curve was normal in these animals and proportionate to that of the goat, when the mean

was much higher a greater part of the curve would have been eliminated, and the area thus removed would have been considerable.

Table V. *Variation in duration of gestation in other species.* (Corrected data.)

Species and breed	Duration of gestation (mean)	Standard deviation	Coefficient of variation	No. of cases	Source of data
Cattle.					
Westerwalder (dairy)	281·4 ±0·1	4·31	1·53	378	Sabatini
Angler (dairy)	282·2 ±0·2	5·41	1·92	268	Sabatini
Oldenberg Wesermarschievh (dual purpose)	279·3 ±0·2	4·74	1·70	304	Sabatini
Shorthorn, Booth (beef)	280·9 ±0·1	1·82	0·65	120	Sabatini
Shorthorn, Durham (beef)	284·7 ±0·1	5·43	1·91	714	Earl Spencer
"Cows"	287·3 ±0·2	8·58	2·99	560	Tessier
Horses.					
Clydesdale (draught)	332·8 ±0·9	10·52	3·16	56	Sabatini
Shires (draught)	333·1 ±0·9	9·43	2·83	50	Sabatini
Kladruber, pure-bred (saddle)	345·4 ±0·3	9·04	2·62	418	Sabatini
Kladruber, cross-bred (saddle)	346·3 ±0·6	9·88	2·85	118	Sabatini
English half-blood Kladruber (saddle)	338·4 ±0·4	8·81	2·60	238	Sabatini
Percheron (draught)	342·2 ±0·4	10·57	3·09	267	Tessier
Rhein-Belgian	332·6 ±0·2	7·21	2·17	1000	Sabatini
Pigs.					
Berkshire	114·78 ±0·08	2·05	1·79	312	Sabatini
Large white	114·72 ±0·08	2·05	1·78	304	Sabatini
Improved Bavarian	114·35 ±0·04	1·85	1·62	322	Sabatini
Hanover-Brunswick	113·2 ±0·1	2·12	1·87	203	Sabatini
Sheep.					
Merino (?)	151·42 ±0·04	1·85	1·22	912	Tessier
Shropshire	145·61 ±0·08	2·22	1·52	358	Sabatini
Hampshire	145·0 ±0·02	2·43	1·68	64	Sabatini
Merino (flesh)	148·97 ±0·08	2·16	1·45	360	Sabatini
Merino (wool)	151·22 ±0·07	1·94	1·28	360	Sabatini
Rhön	150·8 ±0·1	2·54	1·68	140	Sabatini
Man.					
German (from first day of last menses)	280·2 ±0·3	9·16	3·27	536	Collected from Ahlfeld and Schlichting
British (from last day of last menses)	274·0 ±0·3	10·43	3·81	480	Reid

The second method, which was finally adopted, was first to find the mean of the new data and its ratio to the mean of the goat data; then to multiply the corrected range of the goat data by this ratio. This gave the corrected range of the new data which was used for calculating the constants. It was taken to the nearest day on either side of the mean. This method does not allow of the elimination in the cow and horse of possible unrecorded returns of the dam to the male, but as the area of

curve omitted is small and proportionate to that omitted in the curve of the goat data, it is much the better means of correction. Comparisons may easily be made by the use of the coefficient of variation. A summary of these results and the source of the data from which they have been derived is given in Table V.

In horses there is, according to the data available, a well-marked difference between the duration of gestation in saddle horses and draught horses, the larger draught horse having a duration about ten days shorter than the saddle horse. The Percheron occupies an intermediate position. The variability in the two groups is about the same, the saddle horse tending to be less variable. The coefficient of variability is considerably higher than in the goat.

The data on cattle do not permit comparisons; on the whole, the coefficient of variation is low.

The duration of gestation in the Merino sheep is about the same as that of the goat, but in the English breeds it is considerably less. It is noteworthy that the variation is less in the sheep than in the goat, possibly because in the former we are dealing with highly selected breeds. But it must also be remembered that the duration of oestrus is shorter in the sheep, being of the same order or less than that in the cow.

In the pig there is a remarkably small breed difference in the data examined. The coefficient of variation is somewhat less than in the goat.

The figures for man, when corrected for the fact that in one set the commencement of gestation is taken as the first day of the last menses and in the other set as the last day, show very little difference. The coefficient of variation is the highest yet found.

In Table VI the species examined are placed in order of their average coefficient of variation, the least variable being placed first.

Table VI. *The variability of the duration of gestation and of the length of the oestrous period.*

Species	Coefficient of variation	Duration of oestrus*
Sheep	1·47	Few hours to 1 day
Pig	1·77	3 to 4 days
Cow	1·78	6 to 30 hours
Goat	2·16	2 to 3 days
Horse	2·76	3 to 9 days
Man	3·54	23 days (?)

* The durations of oestrus with the exception of that for man are taken from Marshall and Hammond(16).

From this table the interesting fact emerges that the order of variability in the duration of gestation is that of the length of the period

during which coitus is allowed. One hesitates to use the term "oestrus" in this connection owing to the considerable doubt as to the delimitation of this period in man. The pig is an exception to this rule, but the data for this species were also exceptional, when compared with other species, in the small extent of the breed differences. Comparing the figures for different species it seems reasonable to assume that about half the variability is due to this factor.

This result was to be expected, for evidently the time of coitus does not control the time of ovulation, though there is evidence that coitus may influence this time to some extent. Study of the table suggests that about half the variability observed is due to this factor of the length of oestrus. It points strongly to the advisability, when planning work on the causes of parturition, of choosing a species in which the length of oestrus is very short, thus eliminating the variability due to this cause.

In view of the similarity of the curves obtained from the data and the low coefficient of variation in all species, the factors regulating the duration of gestation in the species examined must be regarded as the same. The consensus of opinion is that a series of factors is involved which culminates in the cessation of function of the corpus luteum, a ductless gland regarded as essential for the implantation and nutrition of the embryo. As reviews (Marshall (17), Asdell (18)) have recently been published on this aspect of the question, it is not proposed to enter into details here except to point out that the statistical results obtained lend support to the hypothesis of a single ultimate agent in bringing gestation to a close.

Summary.

(1) A large series of data on the duration of gestation in the goat has been analysed and the constants calculated.

(2) The frequency curve is unimodal and symmetrical, suggesting a single factor or a variety of factors acting simultaneously as the cause for birth.

(3) Slight breed differences exist in the duration of gestation in the goat as in other species examined.

(4) There is a continuous variation in the duration of gestation with the time of year at which conception occurs. Spring conceptions give shorter gestations than autumn conceptions.

(5) There is a distinct difference in the duration of gestation for young and older dams. This is related to the age of the dams and not to the

order of the births. Gestation is shorter in the young animal than in the older.

(6) In the goat the size of litter has little or no effect on the duration of gestation.

(7) Constants for duration of gestation in other species have been obtained. Variability increases with the duration of oestrus and about half the variability in any species with a long oestrous period may be ascribed to this cause.

(8) The factors involved in fixing the duration of gestation in the species examined are evidently the same in all cases, and probably culminate in a single agency responsible for terminating gestation.

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THE AVAILABILITY OF POTASH IN A TYPICAL MAURITIUS SOIL.

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Very considerable amounts of money are annually expended in Mauritius for the purchase of artificial fertilisers, and consequently it is of importance to know what happens to these substances when they are added to the soil. Extensive laboratory experiments have been carried out with nitrogenous fertilisers (1, 2), and also with phosphatic manures (3), and in order to complete the series an investigation into the availability of potash when applied in various forms was undertaken. Local practice favours the use of potash chiefly in the form of nitrate of potash and molasses, and on this account the availability of the potassium oxide in these two substances was tested, and in addition, in potassium sulphate.

When potassic manures are added to the soil, they may behave in two ways: the base may actually enter into combination with the soil complex to form unavailable compounds, or else it may be absorbed.

According to Hissink (4), the absorbed bases form the supply which is immediately available. The process has not only great academic interest, but is also equally important from a practical point of view; thus Page and Williams (5) state that it is of considerable importance in connection with the action of artificial fertilisers in the soil.

Page and Williams (5) have reported experiments regarding the exchangeable potash of the soil, and arrived at the conclusion that there is a gradual conversion of exchangeable potash to a non-exchangeable form, or *vice versa*, depending on whether potash manures are used or not.

The present series of experiments was conducted, therefore, to determine the extent to which the potassium salts when applied to local soils may be converted from an exchangeable form into some other form in which it is no longer available. The availability of the potash in molasses was also tested as well as its effect when applied simultaneously with the potassium salts.

Experimental.

The availability of the potash was tested by means of Dyer's method, and by a modification of the original method of Hissink which, according to Kelley and Brown (6), gives reasonably accurate results. The method of procedure in the latter is as follows: 100 gm. of the soil are treated with 100 c.c. of warm normal ammonium chloride solution and left to stand overnight. The liquid was then poured through a filter paper and the filtrate collected in a 500 c.c. flask. The soil was then treated with further volumes of 100 c.c. of normal ammonium chloride as above, until 500 c.c. of the filtrate were collected. The filtrate was then evaporated to dryness, the ammonium salts driven off at as low a temperature as possible, and the potash determined by the usual methods.

The soil used in this series of experiments was the same as that used in the experiments conducted with the availability of phosphoric acid, the chemical analyses being as follows:

Table I. *Constituents soluble in concentrated hydrochloric acid.*

	% oven dry soil		% oven dry soil
Moisture	0·00	Calcium oxide	0·835
Loss on ignition ...	17·68	Phosphoric oxide ...	0·256
Ferric oxide	21·09	Insoluble matter ...	30·15
Alumina	29·50	Undetermined	0·45
Potassium oxide ...	0·043		

The other determinations made were:

Table II.

1 % citric soluble phosphoric oxide	0·006 % on dry soil
1 % citric soluble potassium oxide	0·0106 „
Exchangeable potassium oxide	0·0093 „
*p*H value	6·4
Calcium carbonate	0·53 %

It is thus seen that this soil is normal from the point of view of chemical analysis of Mauritius soils.

Plan of the experiments.

Six portions of the air dried soil, each of 2 kg., were weighed and intimately mixed with the potash fertilisers according to the following plan:

I. 2 kilos soil + 250 c.c. water.
II. 2 kilos soil + 3 gm. potassium sulphate in 250 c.c. water.
III. 2 kilos soil + 3 gm. potassium nitrate in 250 c.c. water.
IV. 2 kilos soil + 3 gm. potassium sulphate and 60 gm. molasses in 250 c.c. water.
V. 2 kilos soil + 3 gm. potassium nitrate and 60 gm. molasses in 250 c.c. water.
VI. 2 kilos soil + 60 gm. molasses in 250 c.c. water.

The molasses was analysed and found to contain 2·48 per cent. potassium oxide. These differently treated samples were then put into wide-mouthed sample bottles, the mouth being covered with brown paper, and allowed to stand for six months, so that the slow reactions taking place in the soil could reach a state of equilibrium. At the end of this period, analyses were made by the two methods mentioned above to determine (1) the available potash, and (2) the exchangeable potash.

The results of these analyses are given in Table III, the results being calculated on dry soil.

Table III.

		Results obtained by Dyer's method			Results obtained by Hissink's method		
		Available potassium oxide			Exchangeable potassium oxide		
No. of experiment	Total potassium oxide Column A	Theoretical B	By analysis C	Non-available potassium oxide D	Theoretical E	By analysis F	Non-exchangeable potassium oxide G
I.	0·0430	—	0·0106	0·0324	—	0·0093	0·0337
II.	0·1240	0·0916	0·0777	0·0463	0·0903	0·0687	0·0553
III.	0·1130	0·0806	0·0736	0·0394	0·0793	0·0650	0·0480
IV.	0·1984	0·1660	0·1570	0·0314	0·1647	0·1795	0·0189
V.	0·1874	0·1550	0·1491	0·0383	0·1537	0·1790	0·0084
VI.	0·1174	0·0850	0·0951	0·0223	0·0837	0·1147	0·0027

The theoretical available and exchangeable potash is calculated by adding the percentage of potash applied to the dry soil in the various fertilisers or molasses to the percentage of potash obtained in Exp. I, when only water was added. The whole of the potash applied in the manures is soluble, and therefore should be in a perfectly available or exchangeable form.

Comparison between the two methods.

In Table III, Cols. C, D, F and G, the results of the analyses are tabulated so as to show the relationship between the two methods. It will be seen that in general the results given by the two methods agree fairly closely, although as would be expected there are differences in certain respects. Thus in the three experiments where no molasses was added, the citric soluble potash in each case is slightly higher than the exchangeable potash, whereas the reverse is the case where molasses has been added. The reason for this is obscure, but it must be remembered that in each case a point of equilibrium is reached. In the case of the exchangeable potash, the extraction is complete, owing to the fact that successive fresh additions of ammonium chloride are made, whereas with citric acid only one extraction is performed, and therefore all the

citric soluble potash is not extracted. These results show, however, that either method would be equally satisfactory for the determination of probably available potassium oxide in the soil.

THE EFFECT OF THE APPLICATION OF POTASH IN THE DIFFERENT FORMS.

From a consideration of Table III, it is seen that when either potassium nitrate or potassium sulphate is applied to the soil, there is an increase in the non-available and non-exchangeable potash; this is in keeping with Page's results. In both cases, the percentage of added potash which has been changed to the insoluble form is greater in the case of the sulphate than that of the nitrate, the average being 22·5 per cent. for the sulphate and 15 per cent. for the nitrate. A very different state of affairs is noticed when molasses alone had been added to the soil. In this case both the non-available and non-exchangeable potash in the soil has decreased, thus showing that not only has the added potash remained completely available, but that the potassium oxide pre-existing in the soil has also been rendered more available, practically the whole becoming transformed into the exchangeable form. This change is in all probability due to the biological action in the soil. It may possibly be brought about directly by the micro-organisms, or it may be that the decomposition products arising from the sugars, etc., in the molasses attack the stable forms in the soil, and render them available. This effect is best seen from the figures obtained by Hissink's method, although the same thing may be noticed in the results given by Dyer's method to a more limited extent.

The effect of an application of molasses upon simultaneous applications of potassium sulphate and potassium nitrate, respectively, is shown by the results of Exps. IV and V. When these two salts alone are applied, there is an increase in non-available potash, while in the presence of molasses there is a significant decrease, not so great as when molasses alone is used, but still very considerable. The decrease is greater with the nitrate than with the sulphate.

This action of molasses upon the potassium content of Mauritius soil may be of importance, and may be one of the factors explaining the increases in yields of sugar cane which have been shown to follow its application (1, 7) in Mauritius.

THE APPLICATION OF MOLASSES TO MAURITIUS SOILS.

Application of molasses to cane fields was probably practised as early as 1860, but this was far from being a general rule, and even in 1895, Boname (7) stated that the value of this practice was considered by many to be questionable. In 1908, Boname published the results of a series of experiments, showing the beneficial effects of such applications.

In his book *La Canne à Sucre*, de Sornay (8) quotes these experiments and concludes "...ils (the results) nous font voir que l'excédent de rendement n'est pas dû seulement au manque de potasse et d'azote, puisque la différence en faveur de la mélasse avec l'engrais complet est supérieure à celle de l'engrais sans potasse."

Much work has been done locally to seek to explain the action of molasses in the soil. Ebbels and Fauque (9) showed both by laboratory and field experiments that the nitrogen content of treated soils was much higher than in untreated soils, so that they naturally concluded that the increased yield was due to the stimulation of the nitrogen fixation organisms. Later, de Sornay (10) questioned their results and expressed the opinion that the effects were due to biological factors, and that the plant food contained in the molasses is insufficient to account for the whole of the effects. He further suggests that the decomposition products of the molasses in the soil may liberate mineral plant food. Further results are reported by Tempany and Giraud (1), who state: "on the other hand results of de Sornay and ourselves do not indicate that molasses has perceptibly increased the available mineral plant food in soils. Consequently, we conclude that although the decomposition of the molasses may influence to some extent the available plant food content, it is probably not only the only factor in operation." They further stress the importance of biological action, *i.e.* partial sterilisation of the soil following an application of molasses. The possibility of a liberation of plant food from available reserves is admitted, and according to the results obtained in the present series this certainly does occur in regard to the potash content of the soil.

Potash determinations were carried out by Tempany and Giraud after the soil had been leached with tap water once a week for a year, and in this condition no notable increase in available potash had occurred, whilst where sulphate of ammonia was added a probably significant decrease occurred. That no increase, except in one case, occurred is probably due to the fact that the soils were leached at regular intervals, so that it is possible that fairly considerable amounts of potash had been

removed in solution. The decrease in Exp. V is probably significant; in this case only sulphate of ammonia was used, and the solution of this in the soil probably acted as the ammonium chloride in Hissink's method resulting in a displacement of available potash, which has been washed out in drainage water.

Consequently, the results of Tempany and Giraud and of ourselves are not of necessity antagonistic. The results of these experiments show an increase in availability of soil potassium following an application of molasses, and we suggest that if the drainage from the lysimeters had been examined for potash, it would have been found that the largest amounts of potash were obtained from the soils to which molasses had been added.

This increased availability of potash when molasses is applied to the soil may be an extremely important point in soil economy. It must be remembered that the potash content of plants is closely connected with the process of carbon assimilation by the green parts of the plant. It is as a result of this action that carbohydrates are formed in the plant, so that in the absence of other limiting factors, an increase in available soil potash should result in an increased yield of sucrose per acre, when dealing with the sugar cane. It is not suggested that the potash factor is the only one contributing to the beneficial action of molasses, but we do suggest that it is an important one.

It must be borne in mind that increases in yield in sugar cane following applications of molasses similar to those recorded in Mauritius have not been encountered in many other sugar producing countries. It seems reasonable to suppose that the soil conditions in Mauritius may differ from those met with when significant increases are not obtained. The results of this investigation are not, therefore, of general application, but refer only to the lateritic soils found in Mauritius.

Conclusions.

(1) The figures obtained by Dyer's and Hissink's methods show a fairly close agreement, indicating that for the lateritic soils encountered in Mauritius the former method gives reliable data in so far as potash availability is concerned.

(2) When potash salts are applied to these soils, there is a gradual increase in the amount of non-available or non-exchangeable potassium oxide. When equivalent applications of potassium sulphate and potassium nitrate are made, the increase is greater with the former than with the latter.

(3) When molasses is applied to the soil, there is a decrease in the amount of non-available or non-exchangeable potash, showing that the potash in the molasses has remained in the available or exchangeable forms. The decrease is due to the conversion of the non-available forms pre-existing in the soil into available forms.

(4) In the presence of molasses, the potash applied in fertilisers does not revert to an unavailable form, while the potash pre-existing in the soil tends to become more available.

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THE FUNGICIDAL PROPERTIES OF CERTAIN SPRAY-FLUIDS, V.

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FURTHER experiments in testing the fungicidal value of certain spray-fluids, on the lines described in previous communications to this *Journal* (1, 2, 3, 4), were carried out during 1927 and are described below.

Method. The fungus used was *Sphaerotheca Humuli* (DC.) Burr., in the "powdery" conidial stage, on young leaves of rooted cuttings of a certain seedling hop (Ref. No. B. 18), known to be very susceptible to the mildew. Leaves bearing several "powdery" patches of the mildew were sprayed, using a hand "atomiser"; the "control" leaves at the same node, bearing similar patches, were either left untreated or were sprayed with another spray-fluid when a comparison of fungicidal powers was to be made. As in previous experiments, strictly similar biological conditions of fungus and host plant were secured, and it was thus possible to determine within narrow limits the fungicidal value of the spray-fluids.

In order to secure complete wetting of the fungus, a spreader was added to the solutions used.

EXPERIMENTS WITH SPRAY-FLUIDS CONTAINING ARSENIC.

A. *Dicalcium arsenate.*

In previous trials (3, 4) with dicalcium arsenate, difficulty was experienced in obtaining a pure material and the results which were obtained were not entirely concordant. The two preparations then used contained 51·22 per cent. As_2O_5 and 54·91 per cent. respectively. In the experiments described below, a pure monohydrated dicalcium hydrogen arsenate ($CaHAsO_4 . H_2O$) was used, prepared according to the method described in a previous communication (5) dealing with the lime sulphur-calcium arsenate spray; it gave on analysis:

	Percentage	Theoretical
Total calcium oxide (CaO)	28·1	28·3
Total arsenic oxide (As_2O_5)	58·0	58·0

In view of the interaction between this salt and free calcium hydroxide which is usually present in lime casein (calcium caseinate),

it was necessary to use some other spreader. Trials with gelatine[1] at a strength of 0·5 per cent. showed that it brought about a thorough wetting of the patches of mildew and had no fungicidal action.

In Exps. *A* 7, *A* 9 and *A* 15, dicalcium arsenate (with 0·5 per cent. gelatine) at strengths of 0·05, 0·025 and 0·0125 per cent. As_2O_5 proved fungicidal. In Exp. *A* 7, nine leaves were sprayed with the dicalcium arsenate containing 0·05 per cent. As_2O_5, the controls being sprayed with calcium thioarsenate (see Exp. *B* 8) at a strength equivalent to 0·05 per cent. As_2O_5. The effect of the dicalcium arsenate was to kill at once, all the patches showing dead floccoso-collapsed mycelium on the day after application. On eight of the leaves by the sixth day the mildew spots and the underlying tissue were killed right through to the under surface as though cauterised; on the ninth leaf all the patches were killed without discoloration of the mycelium (which remained white) or the death of the underlying tissue. No scorching was caused to the leaf tissue except to a slight extent on one leaf. All the control leaves were seriously injured by the calcium thioarsenate (see Exp. *B* 8). In Exp. *A* 9 nine leaves were sprayed with a solution containing 0·025 per cent. As_2O_5, the controls being sprayed with a solution of calcium thioarsenate equivalent to 0·025 per cent. As_2O_5. On six of the leaves sprayed with dicalcium arsenate the mildew patches and underlying leaf tissues were killed with the cauterising effect noted above, while this effect was absent or only little evident on three leaves. Slight scorching injury was caused to four leaves at their margins. All the control leaves were more seriously scorched (see Exp. *B* 10). In Exp. *A* 15 twelve leaves were sprayed with a solution containing 0·0125 per cent. As_2O_5, the control leaves being sprayed with calcium thioarsenate solution equivalent to 0·0125 per cent. As_2O_5. The solution proved fungicidal; on six leaves the mildew patches were killed without any cauterising effect, on four leaves this effect was just visible and on two it was clearly evident. Only one leaf showed (at the tip) slight injury due to scorching; several of the control leaves showed serious scorching injury (see Exp. *B* 16).

In four experiments (*A* 21, *A* 51, *A* 54, *A* 60) dicalcium arsenate solution containing 0·006 per cent. As_2O_5 (with 0·5 per cent. gelatine) was used. In Exp. *A* 21 twelve leaves were sprayed, the control leaves being sprayed with a solution of calcium thioarsenate (0·006 per cent. As_2O_5 with 0·5 per cent. gelatine). The dicalcium arsenate proved fungicidal, the mildew patches on all the leaves being sterile on the ninth

[1] The gelatine used was Coignets gold label No. 1 brand.

day after spraying. No cauterising effect was produced and no injury was caused. Some of the control leaves showed injury due to scorching (see Exp. *B* 22). In Exp. *A* 51 seven leaves were sprayed. By the thirteenth day the mildew patches were dead or dying and mostly sterile; on five leaves scattered or isolated conidiophores were visible at the edge of a few patches and on two leaves a few clustered conidiophores occurred at the edge of several patches. The solution appeared to be almost, but not quite, fungicidal. In Exp. *A* 54 seven leaves were sprayed with a solution of dicalcium arsenate containing 0·006 per cent. As_2O_5 with 0·5 per cent. gelatine and the control leaves (Exp. *A* 53) with the same solution to which 0·3 per cent. of hydrated lime[1] was added. In Exp. *A* 54 many of the patches on all the leaves were killed; on three leaves a few scattered or isolated conidiophores were produced by the thirteenth day at the edge of a few patches and on four leaves several patches produced minute clusters of conidiophores. It appeared again (see above Exp. *A* 51) that a solution of dicalcium arsenate containing 0·006 per cent. As_2O_5 was just below fungicidal strength. In Exp. *A* 53 where 0·3 per cent. hydrated lime was added the result was entirely different; by the third day after spraying all the patches showed a sparse fresh growth of scattered conidiophores and by the thirteenth day all the patches were densely powdery with clustered conidiophores. It was clear that by the addition of the hydrated lime an almost fungicidal solution was rendered non-fungicidal. In Exp. *A* 60 eight leaves were sprayed with a solution of dicalcium arsenate containing 0·006 per cent. As_2O_5 (and 0·5 per cent. gelatine) and the control leaves (Exp. *A* 61) with the same strength of dicalcium arsenate and 1 per cent. lime casein[2] in the place of the 0·5 per cent. gelatine. In Exp. *A* 60 the solution proved fungicidal in some cases and almost so in other cases; on five of the eight leaves all the patches were killed, on the remaining three leaves a very few clustered conidiophores were produced at the edge of a few of the youngest patches. In Exp. *A* 61 the effect of the use of 1 per cent. lime casein with the dicalcium arsenate solution was to render it completely non-fungicidal; all the patches on all the leaves were again densely powdery by the tenth day. In Exp. *A* 57 eight leaves were sprayed with a solution of

[1] The hydrated lime employed was a commercial hydrated lime containing 89·5 per cent. free calcium hydroxide and 5·96 per cent. calcium carbonate.

[2] Lime casein (calcium caseinate) was prepared from 12·5 gm. commercial casein and 37·5 gm. commercial hydrated lime. These materials were mixed with a small quantity of water and finally made up to 1 litre to obtain a 5 per cent. suspension. The 1 per cent. dilution therefore contained approximately 0·75 gm. hydrated lime.

dicalcium arsenate containing 0·006 per cent. As_2O_5 plus 0·75 per cent. hydrated lime and 0·5 per cent. gelatine and the control leaves (Exp. *A* 58) with the same strength of dicalcium arsenate plus 1 per cent. lime casein. In each of these last two experiments the solution proved to be non-fungicidal.

Dicalcium arsenate at a strength of 0·003 per cent. As_2O_5 (and 0·5 per cent. gelatine) was used in one experiment (*A* 27). Seven leaves were sprayed. Considerable fungicidal power was shown, for many of the patches remained sterile and were presumably killed; at the edges of some of the patches however a few scattered conidiophores were produced by the fourteenth day.

The results of the above experiments show that dicalcium arsenate (monohydrated dicalcium hydrogen arsenate) at a strength of 0·0125 per cent. As_2O_5 was fungicidal, while at 0·006 per cent. As_2O_5 it was not quite fungicidal. In previously recorded experiments it was found in one series (3) that dicalcium arsenate containing 0·024 per cent. As_2O_5 was apparently just fungicidal and at 0·048 per cent. As_2O_5 was certainly fungicidal. It will be seen therefore that in later work dicalcium arsenate at a lower strength has proved fungicidal. This different result is undoubtedly due to two factors, firstly, the samples of calcium arsenate that were used in the earlier work were more basic than the dicalcium hydrogen arsenate used in the above recorded experiments. Secondly, to the reaction between the calcium arsenate and free lime present in the 1 per cent. lime casein added as a spreader in the earlier experiments, a reaction resulting in the production of a still more basic calcium arsenate.

The effects of the reaction between dicalcium arsenate and lime are clearly shown in Exps. *A* 53, *A* 57 above, where the addition of hydrated lime caused a diminution in the fungicidal power of the arsenate and in Exps. *A* 58, *A* 61, where lime casein containing free lime was used in place of gelatine.

It appears therefore established that the immediate fungicidal action of the calcium arsenates depends upon the amount of arsenic present in solution. The addition of free lime which causes a temporary precipitation of the soluble arsenic brings about a loss of fungicidal properties, but, as has been shown (5), the action of the carbon dioxide of the air is ultimately to remove the excess of lime in the form of calcium carbonate and to restore the dicalcium arsenate.

It may also be pointed out here that the results previously obtained in the case of disodium hydrogen arsenate (3) were possibly influenced

in a similar manner by the addition of 1 per cent. lime casein as a spreader.

B. *Calcium thioarsenate.*

In a communication dealing with the chemistry of the mixed lime sulphur-lead arsenate spray (6) it was suggested that additional fungicidal properties might be expected from the presence of soluble arsenates and thioarsenates and it has also been shown (4) that the fungicidal strength of the mixture is greater than that of either of its constituents. Amongst the experiments then recorded was one with lead thioarsenate which was shown to possess high fungicidal powers. As this lead thioarsenate was not soluble in water and the greater fungicidal power of the mixed spray was found to lie in the filtrate, it became evident that soluble calcium arsenates and thioarsenates were most probably responsible for the increased fungicidal properties.

It was therefore of interest to ascertain whether the association of sulphur with arsenic in the calcium thioarsenates would confer a greater fungicidal power than is shown by the arsenic of the calcium arsenates themselves.

For the purpose of this experiment attempts were made to prepare certain calcium thioarsenates with which the fungicidal power of dicalcium hydrogen arsenate could be compared. Although well-defined salts could not be isolated owing to their instability and the readiness with which sulphur separated when efforts were made to crystallise them, mixtures of the various dicalcium thioarsenates were prepared. Relatively stable solutions were found to be produced by the interaction of sulphuretted hydrogen and dicalcium hydrogen arsenate. This interaction was brought about by passing the gas into the suspension of dicalcium hydrogen arsenate for several hours and then heating in a stoppered pressure bottle for three hours in boiling water. It may be mentioned here that during treatment with sulphuretted hydrogen an increased amount of the dicalcium arsenate goes into solution, indicating that the calcium thioarsenates are more soluble than the dicalcium arsenate itself. After cooling and filtering, the resultant solution was reserved for analysis and stored in the dark.

Two such solutions were employed:

Calcium thioarsenate I, which gave on analysis:

100 c.c. contain 0·229 gm. arsenic as As_2O_5 and 0·0807 gm. sulphur. The molecular ratio of arsenic oxide to sulphur, $As_2O_5/2 : S = 1 : 1{\cdot}27$, indicates the preponderance of the dicalcium hydrogen monothioarsenate.

Calcium thioarsenate II, which gave on analysis:

100 c.c. contain 0·545 gm. arsenic as As_2O_5 and 0·556 gm. sulphur. The molecular ratio $As_2O_5/2 : S = 1 : 3{\cdot}67$ indicates the presence of higher thioarsenates than in the case of solution I.

The two preparations were used in a number of experiments recorded below, in each case 0·5 per cent. gelatine being added as a spreader.

Preparation I. This was used at strengths equivalent to 0·05, 0·025, 0·0125, 0·006 and 0·003 per cent. As_2O_5. In Exp. *B* 8, at a strength equivalent to 0·05 per cent. As_2O_5, the solution seriously injured the healthy tissue at the margins of the sprayed leaves, besides killing the tissue where the mildew patches occurred. The control leaves sprayed with dicalcium arsenate of the strength of 0·05 per cent. As_2O_5 showed practically no scorching injury (see Exp. *A* 7). In Exp. *B* 10 nine leaves were sprayed with calcium thioarsenate and the controls with dicalcium arsenate, the solution in each case being equivalent to 0·025 per cent. As_2O_5. The calcium thioarsenate was fungicidal, the mildew patches being killed as though cauterised and on seven of the leaves serious scorching was produced, usually near the margin. The scorching injury was decidedly more severe than that which was caused by the dicalcium arsenate (see Exp. *A* 9). In Exp. *B* 16 twelve leaves were sprayed with calcium thioarsenate and the controls with dicalcium arsenate, each solution being equivalent to 0·0125 per cent. As_2O_5. The calcium thioarsenate was fungicidal; on the younger leaves the patches were usually killed with no cauterising effect and no scorching of the leaf tissue occurred; on the older leaves more or less serious scorching injury resulted and usually a cauterising effect was noticeable on the mildew patches. The control leaves sprayed with dicalcium arsenate showed practically no scorching injury (see Exp. *A* 15). In Exp. *B* 22 twelve leaves were sprayed with calcium thioarsenate equivalent to 0·006 per cent. As_2O_5 and the controls with dicalcium arsenate of the same strength. The calcium thioarsenate proved fungicidal; no scorching injury appeared on nine of the leaves, while the injury was slight on one leaf and severe on two. On the control leaves no scorching injury was caused (see Exp. *A* 21). In Exp. *B* 28 seven leaves were sprayed with calcium thioarsenate and the controls with dicalcium arsenate, the solution in each case being equivalent to 0·003 per cent. As_2O_5. The calcium thioarsenate proved fungicidal to all the patches on three leaves, but from some of the patches on the other four leaves a few scattered conidiophores had been produced by the fourteenth day after spraying. No scorching injury was produced. The same fungicidal power was shown

by the dicalcium arsenate on the control leaves (see Exp. *A* 27). In Exps. *B* 29 and *B* 30 eight leaves were sprayed with calcium thioarsenate I and the control leaves with calcium thioarsenate II, in each case the strength being equivalent to 0·003 per cent. As_2O_5. No difference in fungicidal action was observed; on five of the sprayed leaves some of the mildew patches remained sterile and presumably were killed, while others produced a few clustered conidiophores at the edges; on three of the leaves several of the patches produced denser clusters of conidiophores, showing clearly that the preparations were non-fungicidal at the strength used.

Preparation II. Besides being used at a strength equivalent to 0·003 per cent. As_2O_5 (see Exp. *B* 30 above), this preparation was used in two other experiments, where eight leaves were sprayed with a solution equivalent to 0·0125 per cent. As_2O_5 (Exp. *B* 23) and the control leaves with a solution equivalent to 0·006 per cent. As_2O_5 (Exp. *B* 24). In both the experiments the preparation proved fungicidal and no scorching injury was produced. The mildew patches were killed at once with a very marked cauterising effect; the underlying tissue was also killed, extending sometimes, but not always, to the lower surface of the leaf.

From the above experiments it can be stated that calcium thioarsenate II is fungicidal and causes no scorching injury at a strength equivalent to 0·006 per cent. As_2O_5, and that at a strength equivalent to 0·003 per cent. As_2O_5 preparations I and II are both below fungicidal strength. The preparation I, which contained a smaller proportion of sulphur, used at the same strength as preparation II caused apparently more scorching injury (compare Exps. *B* 16, *B* 22 with *B* 23, *B* 24). It is clear also that calcium thioarsenate is more liable to cause scorching injury than dicalcium arsenate.

The results of the above tests show further that both of the preparations of calcium thioarsenate have a greater fungicidal power than the lead thioarsenate used in previous experiments (4). The lead thioarsenate, unlike the calcium thioarsenates, was not soluble in water and could only be used in suspension. It behaved therefore similarly to lead arsenate which has been shown (4) to possess a much smaller fungicidal power than the soluble dicalcium arsenate.

Owing to the fact that none of the thioarsenates used in these experiments was quite pure, it is not desirable to draw definite conclusions as to the part played by sulphur in the fungicidal activity of the thioarsenates. The calcium thioarsenates have shown fungicidal

properties superior to the dicalcium arsenate and as they are formed when lime sulphur is mixed with lead arsenate or calcium arsenate, they must be regarded as being one of the main factors in the production of the increased fungicidal power of the mixed spray.

Whilst it is not impossible that lead thioarsenate is formed and plays a part in the lime sulphur-lead arsenate spray, the view is favoured that it is rather the calcium thioarsenates together with calcium arsenates which are responsible for the increase in fungicidal power.

SUMMARY.

(1) A solution of dicalcium hydrogen arsenate at a strength of 0·0125 per cent. As_2O_5 was fungicidal to the conidial stage of *Sphaerotheca Humuli*, while at 0·006 per cent. As_2O_5 it was not quite fungicidal.

(2) Lime casein containing calcium hydroxide when added as a spreader to calcium arsenate was shown to reduce the fungicidal properties of the calcium arsenate spray.

(3) A solution of calcium thioarsenate at a strength equivalent to 0·006 per cent. As_2O_5 was fungicidal to the above fungus, while at 0·003 per cent. As_2O_5 it was below fungicidal strength.

(4) It is suggested that the increased fungicidal properties of the mixed lime sulphur-lead arsenate spray are due to the presence of calcium thioarsenates.

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THE ANALYSIS OF TOMATO PLANTS. PART I[1].

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(With Two Text-figures.)

Introduction.

As a result of manurial experiments carried out at this station during the last decade or so much is now known of the effects of potassic and nitrogenous fertilisers on the tomato plant. The rôle of phosphatic fertilisers, however, is still a matter of doubt. On this particular soil, phosphates appear to be without effect on crop weight or the appearance of the plant. The evidence accumulated will be considered elsewhere, but the effect of phosphates on the weight of tomato fruit will be appreciated from Table I which is compiled from the Annual Reports of this station (2).

Table I. *Crop produced (lb. per plant) by tomato plants receiving varying amounts of phosphates—nitrogen and potash being approximately constant.*

		Phosphates applied calculated as $Ca_3(PO_4)_2$				
Year	Complete artificials	None	8 %	16 %	24 %	32 %
1923	5·09	5·01	4·43	4·59	4·36	—
1924	6·12	5·87	5·86	5·87	6·31	—
1925	5·34	5·77	—	5·47	5·43	5·50
1926	3·84	4·31	4·23	4·09	4·29	—

In the years 1923 and 1924 the variety Comet was grown in these experiments. In 1925 and 1926 the variety was Manx Marvel. Previous to 1923 none of these plots had received phosphatic manures for some years.

Examination of the table shows that the effect due to the inclusion of phosphates in the fertiliser is within the limits of experimental error. Details of the time of picking and the quality of the fruit show that phosphates are without effect on maturity and quality. Further, it may be remarked that examination of plants in another house in two plots receiving respectively complete artificials and complete artificials without phosphates fails to reveal any difference in the appearance of the plants.

[1] Part of a thesis approved for the degree of Doctor of Philosophy in the University of London.

Little work seems to have been done on this subject in England but these findings are, in general, in contradiction to those arrived at by American workers. Most of the results show that the addition of phosphates in some form or another is essential in tomato cultivation. It is not proposed to consider the numerous papers which have dealt with this subject but mention must be made of one by MacGillivray (3) who has published an account of a detailed study of the effect of phosphorus on the tomato plant. Using sand cultures and tomatoes of the Bonny Best variety, he determined the effects of no-phosphorus and ample-phosphorus supplies. Although sand as a medium is not strictly comparable with soil his results indicate clearly the ill-effects which followed the omission of phosphorus. The dry matter produced was very low as was the phosphorus content of the plants. Single fruits were prevalent on the trusses and, although there was no effect on the quality of the seed, omission of the phosphorus was associated with a reduction in the number of seed per fruit. Definite effects were also produced on the stems and foliage.

The discrepancy between American results and those given in Table I made some enquiry desirable. Of the possible lines of investigation it was considered that analysis of plants with respect to potash, phosphoric acid and nitrogen seemed most promising. From such analyses it was hoped that, firstly, the amounts of these three nutrients removed from the soil by a tomato crop could be determined under typical English conditions; secondly, the analyses would show whether the intake of the nutrients had any appreciable mutual effect; thirdly, by analysing plants from manured and unmanured plots respectively the effect of manuring on the composition of the plants would be determined.

Preliminary analyses of typical tomato foliage from various plots showed real differences in composition. Analysis of plants grown in completely manured plots showed that the ratio potash (K_2O) : phosphoric acid (P_2O_5) : nitrogen was of the order 9 : 1 : 5. The difference between this ratio and that obtaining in a widely used "complete" tomato fertiliser, namely, 0·62 : 1 : 0·51, confirmed the views that work on these lines would prove of value.

Accordingly, analyses were made during the 1925 season of plants grown in soils receiving respectively complete manurial treatment and no manure. In the 1926 season more detailed information was sought by the analyses of plants in another house.

EXPERIMENTAL.

(A) *Cultural.*

The two plots in which the 1925 plants grew are part of manurial experiments which have been in progress for some years. They are designated K 2 and K 3. K 2 has been unmanured since 1917 with the exception of an annual dressing of hydrated lime at the rate of one pound per square yard. K 3 has been manured regularly and for the 1925 season was dressed as follows: before planting, pounds per square yard, hydrated lime 1·0, stable manure 14, basic slag 0·4, bone meal 0·5, bone flour 0·5, ground hoofs 0·5, sulphate of potash 0·6; during the season a top-dressing was applied in eight equal instalments and amounted to, in pounds per square yard: superphosphate 0·5, sulphate of potash 0·2, sulphate of ammonia 0·2, nitrate of soda 0·2.

In 1926 plants grown in house M were used. This house is arranged for variety trials and has always been well manured. For the season in question the manurial treatment was similar to that already given for the plot K 3 in 1925.

Some analytical data for the soils are given in Table II.

Table II.

	K 2 (1925)	K 3 (1925)	M (1926)
Total nitrogen	0·233	0·319	0·394
Total potash (K_2O)	0·258	0·440	0·600
Total phosphoric acid (P_2O_5)	0·437	0·940	1·156
Soluble in 1 % citric acid:			
Potash	0·0039	0·097	0·062
Phosphoric acid	0·090	0·129	0·140

The houses run north and south and the plants are grown in rows at right angles to the length of the house. The plants are arranged 1 ft. 1 in. apart in the rows and the rows are arranged with alternate distances of 1 ft. 6 in. and 2 ft. 3 in. between them. In 1925 the experimental plants numbered 19 and consisted of two adjacent rows in each plot. The rows were selected away from the edges of the plots so that, as far as possible, they should be representative of that particular plot and not be influenced by the manurial treatment and growth in any adjoining plot. The plants were treated throughout the season in the usual commercial manner with the exception that those in K 2 received no manure. All trimmings from the plants were brought to the laboratory immediately and prepared for analysis as soon as possible. All fruit was picked when ripe and representative samples analysed.

The plants analysed in 1926 were grown in the house M and the arrangement of the plants was the same as that already described for the plots in house K. The following modification of the procedure described was followed in handling the material. Instead of allowing all the plants to grow to maturity they were harvested at different times. In this way the rate of growth and the changes in composition during development were followed. In addition the fruit from each truss of each plant was dealt with separately. Two rows of twenty plants each were used. These were divided into eight groups of five plants each. The groups were numbered 1 to 8 and the plants in each group labelled *a*, *b*, *c*, *d*, *e*. It was intended that the plants *a*, *b*, and *c* in each group should be analysed and that the *d* and *e* plants should constitute reserves to be used in the event of loss of any of the plants. Each group was to be harvested and analysed at the end of successive months, beginning with Group 1 at the end of March and ending with Group 8 at the end of October. Unfortunately it was not possible to carry out this programme in its entirety. Owing to the shortage of coal at the end of the season it was impossible to heat the houses during September and October when the plants obviously required heat. Consequently the plants in Groups 7 and 8 were harvested at the end of September.

It may be contended that three plants are insufficient for such work. In reply it may be stated that when the plants have grown to a height of 4 ft. or more they are unwieldy, and, in a laboratory of ordinary equipment, it is difficult to deal with more than three such plants at a time.

In 1926 as in 1925 the usual commercial practice was followed in cultivation. All the trimmings from each plant were dried separately and saved till the plants were harvested, and the same procedure was adopted for the fruit from each truss from each plant.

(B) *Analytical.*

Immediately after arrival in the laboratory the material was carefully freed from any particles of foreign matter and the green weight recorded. The samples were then dried to constant weight in an oven maintained at 98 to 100°. Generally constant weight was attained in twelve to sixteen days. The dried tissue was then ground in a porcelain mortar until all passed a fine sieve when it was deemed ready for analysis. In the case of old plants it was necessary to cut up the dried stalks prior to grinding in a hand mill but in all cases sieving was resorted to before analysis.

The percentages of nitrogen and ash were determined in the dry tissue and the ash analysed for potash (K_2O) and phosphoric acid (P_2O_5).

The nitrogen estimations were carried out by the Kjeldahl method, 0·75–1 gm. of the dry tissue being used for each determination. Good agreement was obtained in all duplicate determinations. This points to the homogeneity of the material and suggests that the amount of nitrate nitrogen present and of compounds to which the Kjeldahl method is not applicable is negligible.

For the ash estimations 2–3 gm. were heated at a low red heat to constant weight. Throughout this, and subsequent work of a similar nature, porcelain crucibles were used and with the precautions mentioned below they proved satisfactory. After a few preliminary determinations it became fairly easy to decide when combustion of foliage and stems was complete from the colour of the ash. At constant weight the ash was of a uniform grey colour. When combustion was incomplete the colour varied from darker shades of grey to black. Ashing of the fruit could not be associated with any change of colour, various shades of grey and occasionally traces of black being shown. The black material was carbon but the amount was negligible as was seen during the subsequent solution of the ash in acid. During the preliminary determinations it was noticed that ashing the fruit at a high temperature caused the material to fuse with the evolution of bubbles, presumably carbon dioxide. This always resulted in inconsistent results and a low ash content. Consequently, great care was taken to work at such a temperature that the crucibles were never above a low red heat.

For the estimation of the two ash constituents the weighed ash was dissolved in 10 per cent. hydrochloric acid. It was noticed that during the solution of the ash of green tissue hydrogen sulphide was invariably evolved. The acid solution was brought to the boil and filtered into a graduated flask. After washing the insoluble matter the filtrate was cooled and made up to the known volume. Aliquot parts were measured into silica dishes, sufficient baryta solution added to precipitate the sulphates and also some pure calcium carbonate. Then followed the usual treatment to render silica insoluble. Potassium in the hot aqueous extract of the residue was estimated by the perchlorate method, and phosphoric acid in the acid extract was precipitated as ammonium phosphomolybdate and eventually weighed as the blue anhydride.

The results of the analyses in 1925 are shown in Tables III and IV. The results for 1926 are detailed in Tables V to IX.

Table III. *The weights in gm. and analyses of trimmings, fruit, etc. from plants in* K 3—*manured*—1925.

Date of picking (1925)	Green weight	Dry weight	In dry matter: Ash %	N %	K_2O %	P_2O_5 %	Total ash	Total K_2O	Total P_2O_5	Total N
At planting	136·61	9·553	18·64	4·081	8·404	1·103	1·781	0·8028	0·1054	0·3897
21. iv.	245·5	23·28	14·50	5·779	4·467	1·029	3·368	1·004	0·2396	1·346
28. iv.	250·7	22·96	30·83	4·162	8·642	0·8445	7·877	2·308	0·225	1·064
4. v.	39·6	3·68								
12. v.	515	44·03	41·00	3·221	12·23	0·7263	18·05	5·385	0·3198	1·421
19. v.	240	20·12	36·00	2·733	9·759	0·4752	7·243	1·963	0·0956	0·550
16. vi.	614·4	53·08	41·00	2·787	7·743	0·6213	21·75	4·110	0·3295	1·479
3. vii.	1,480	143	30·70	2·997	8·226	0·4687	43·89	11·76	0·6701	4·285
29. vii.	5,095	543	28·56	3·114	7·884	0·4692	155·1	40·64	2·548	16·91
26. viii.	5,436	550	27·49	3·244	7·145	0·4789	151·2	39·30	2·634	17·84
16. ix.	3,451	325	26·34	4·241	7·593	0·7025	85·63	24·68	2·283	13·78
23. x.	11,493	976	26·42	5·022	7·565	0·7745	257·8	73·81	7·734	49·00
Total green parts	28,860	2704·15	—	—	—	—	751·908	204·96	17·0786	107·675
Fruit	46,117	2838·32	13·02	2·448	4·980	0·7758	369·6	141·4	21·45	69·48
Total	74,977	5542·47	—	—	—	—	1121·508	346·36	38·5286	177·155
Average per plant	3,947	291·7	—	—	—	—	59·02	18·22	2·028	9·324

Table IV. *The weights in gm. and analyses of trimmings, fruit, etc. from plants in* K 2—*unmanured*—1925.

Date of picking (1925)	Green weight	Dry weight	In dry matter: Ash %	N %	K_2O %	P_2O_5 %	Total ash	Total K_2O	Total P_2O_5	Total N
At planting	136·61	9·553	18·64	4·081	8·404	1·103	1·781	0·8028	0·1054	0·3897
21. iv.	167·2	15·64	18·56	5·105	2·491	1·089	2·891	0·3896	0·1704	0·795
28. iv.	207	18·51	30·46	2·951	2·775	0·5618	5·771	0·5137	0·1004	0·5462
12. v.	550	52·96	35·11	2·646	1·629	0·6950	18·60	0·8628	0·3682	1·402
19. v.	70·54	8·47	34·39	2·964	1·321	0·7365	21·16	0·8134	0·4534	1·825
16. vi.	568·4	53·08								
3. vii.	810	78·00	31·54	2·882	1·619	0·4849	24·60	1·263	0·3782	2·253
29. vii.	699	106·5	30·84	2·795	1·361	0·6504	32·78	1·450	0·6928	2·977
16. ix.	1,812	428	35·42	2·979	1·945	0·7377	148·1	8·325	3·157	12·75
23. x.	5,716	800	22·06	2·737	2·966	0·4555	176·5	23·73	3·644	21·92
Total green parts	10,600	1561·16	—	—	—	—	430·402	37·3475	8·9644	44·4682
Fruit	27,376	1414·4	5·91	3·468	1·091	0·6952	83·66	15·425	9·836	49·06
Total	37,976	2975·56	—	—	—	—	514·062	52·7725	18·8004	93·5282
Average per plant	1,998	156·6	—	—	—	—	27·06	2·775	0·9895	4·922

DISCUSSION OF THE RESULTS.

For convenience the results for the two years will be considered separately. In connection with those for 1925 several points of general interest stand out as worthy of mention.

From Table III, which gives the results for the manured plot K 3, it will be seen that a marketable crop of 46,117 gm. of fruit requires for its production 28,860 gm. of green material. The ratio of these quantities is approximately 1·6 to 1. Obviously the grower wants to keep this ratio as low as possible and it would be of interest to determine its value under different sets of commercial conditions. In the present instance the crop amounted to almost 5·5 lb. of fruit per plant. It is safe to say that in a house twelve years old, and which has been in regular cultivation, this is a very satisfactory crop: We can, therefore, assume that under the conditions prevailing in the nursery here, a good crop requires the production of about two-thirds of its weight of green material. In passing, it may be noted that the grower rarely has difficulty in encouraging excessive vegetative growth but such growth is not, as a rule, accompanied by a satisfactory crop. The attempt is always made to produce compact bushy plants.

The crop of 46,117 gm. entails the removal from the soil of 346·36 gm. of potash, 38·53 gm. of phosphoric acid and 177·16 gm. of nitrogen. Of these amounts 204·96 gm. of potash, 17·08 gm. of phosphoric acid and 107·68 gm. of nitrogen are contained in the green parts and the remainder in the fruit. So that rather more than half of the total nitrogen and two-thirds of the total potash is used to build up the structure that carries the fruit. Under these conditions a 40 ton crop of tomatoes per acre would necessitate the removal of 672 lb. of potash, 75 lb. of phosphoric acid and 344 lb. of nitrogen per acre each year. Since a crop of this weight is a good average it is probable that these figures are in the region of the maximum annual removal of the three nutrients. The figures are of significance if compared with the amounts of these nutrients which are added in the form of manures each year.

The phosphorus requirements of the tomato plant.

The low intake of phosphorus in the manured plot K 3 is outstanding. Several reasons may be advanced to account for this. In the first place it may be indicative of the difficulty of the plant to absorb phosphorus. Russell (4) quotes the case of the turnip which has a low phosphorus content but which reacts to phosphatic fertilisers. As has been shown

in Table I tomatoes appear to be unaffected by the omission of phosphates as judged by the weight of fruit produced. A second possibility is that the absorption of phosphorus is influenced by the absorption or the concentration in the soil of potash and nitrogen. Although the soil in the plot K 3 was amply supplied with potash, nitrogen and phosphates the relative amount of phosphorus taken up by the plants was less than that taken up by the plants in the unmanured plot K 2. It is probable, therefore, that there is some interdependence between the concentration of the three nutrients in the soil and the amounts absorbed by the plants. From data to be reported in a second paper it appears that there is a definite relation between soil phosphates and the absorption of potash and between the soil potash and the absorption of phosphoric acid. The third possibility is that the absolute phosphorus requirements of the tomato plant under our conditions are low. The data presented here and the results of repeated manurial trials suggest that this, in part at all events, is the probable explanation.

The ratio of the three nutrients in the manured plant.

From Table III it will be seen that the ratio $K_2O : P_2O_5 : N$ in the manured whole plants is 8·98 : 1 : 4·60. This is much at variance with the ratio of the same materials in a widely-used tomato fertiliser, namely, 0·62 : 1 : 0·51. The reason for the high phosphate content of English tomato fertilisers is difficult to understand. It seldom happens that applications of phosphates affect the plants, although it is widely believed among growers that phosphates "harden" plants. This is easily attained by dressings of sulphate of potash, and it is our experience that where growers have reduced their usual phosphate dressings the resulting crops have not been below standard.

The variation in composition of foliage on different dates.

Consideration of the detailed figures brings out several important facts. All the samples under the various dates were of the green parts of the plants. The early samples consisted of side-shoots. During the middle period the samples consisted chiefly of leaves and in August and September they were leaves and shoots. The samples taken on 23. x. 25 consisted of all the aerial parts of the plants as they were growing at the time.

In general the values for nitrogen and phosphoric acid fall to a minimum during May and then rise again. Except for the value for potash on 28. iv. 25 the same holds for that nutrient also.

These results are in general agreement with the findings of other workers who conclude that the growing points of tomato plants are always richest in these nutrients. In the present analyses the high values obtaining in the early part of the season are due to the side-shoots. Later, the proportion of leaves, which are always old, increases and the values fall. Later still, the proportion of side-shoots increases and the final sample contained fairly big side-shoots and these constituted an appreciable part of the total weight. Consequently, we have the high values for this sample.

The results for the unmanured plot in 1925.

The results for the unmanured plot K 2 are given in Table IV.

In this case the crop amounted to 27,376 gm. or approximately 3·2 lb. per plant. Such a crop is below the level of economic production. This crop necessitated the production of 10,600 gm. of green matter. The ratio of fruit to green matter is approximately 2·6 to 1. Compared with the figures for K 3 it appears, at first sight, that the plants have been functioning more efficiently despite the lack of manures. Actually this is not the case—from the commercial standpoint. Apart from a difference of at least 2 lb. per plant in the crop, the proportion of first quality fruit in the K 2 plants was only 43·25 per cent. of the total crop, whereas in the K 3 plants it was 94·38 per cent. So that the issue is confused by the quality factor. Some light is thrown on this point by the analytical figures for the fruit in the two cases. They are as follows, being the percentages on the weight of the fruit when picked:

	H_2O	K_2O	P_2O_5	N
K 2	94·9	0·0556	0·0355	0·1769
K 3	93·5	0·3237	0·0491	0·1591

The difference in the nitrogen content in the two cases is small. The phosphoric acid, however, is over 40 per cent. higher in the fruit of the plants in the manured plot K 3, and the potash in the fruit of the same plants is almost six times as much as that in the fruit obtained from the unmanured plot K 2. This points to potash being the predominant factor in determining the quality of the fruit. The fruit from the plot K 2 contained 43·25 per cent. of blotchy fruit which had ripened irregularly. Bewley and White (1) find that this blotchy ripening is "the result of malnutrition in respect of potash and nitrogen, especially the former." From the figures given above it is clear that the K 2 fruit was

not deficient in nitrogen. The low potash content of the fruit in K 2, then, is probably responsible for the poor quality of the fruit.

It would appear therefore, that the ratio fruit : green matter will only determine the efficiency of the plant as a fruit producer when the weight of fruit is reasonably high and the quality good.

The crop of 27,376 gm. produced in the unmanured plot necessitated the removal from the soil of 52·78 gm. of potash, 18·80 gm. of phosphoric acid and 93·53 gm. of nitrogen. The difference between these values and those for K 3 shows what an effect manuring has on the plant.

The total dry matter produced in K 2 was 156·6 gm. per plant, as against 291·7 gm. in K 3, that is, manuring has practically doubled the dry matter produced.

As was to be expected there is a decided difference in the ratio $K_2O : P_2O_5 : N$ for the whole plants in the two cases. Whereas in K 3 it was 8·98 : 1 : 4·60 here it is 2·8 : 1 : 5.

The amount of nutrients removed per plant.

The removal in gm. is as follows:

	K_2O	P_2O_5	N
K 3 (manured)	18·22	2·03	9·32
K 2 (unmanured)	2·78	0·989	4·92

The feature of these figures is the fall in the amount of potash in the unmanured plants as compared with that of the plants in the manured plot. Whereas the nitrogen and phosphoric acid removed are approximately halved the potash is reduced to less than a sixth where the manure was omitted. Several explanations of this are possible and the following is suggested. The unmanured soil is deficient in potash and phosphoric acid. Because the potash concentration is low the phosphoric acid content of the plants tends to be high and because the concentration of phosphoric acid in the soil is low the potash content of the plants tends to be low. These effects which act together in the unmanured soil depress the $K_2O : P_2O_5$ ratio in the plants. Hence, compared with the phosphoric acid value, the potash value for the unmanured plants is lower than that for the manured plants. The suggestion of an interdependence between soil potash and phosphoric acid on the one hand and the phosphoric acid and potash contents of the plants on the other is supported by data to be presented and discussed in a later paper.

The distribution of the nutrients between the fruit and vegetative parts.

A comparison of potash, phosphoric acid and nitrogen in the plants of both soils is given in the following table:

	K_2O		P_2O_5		N	
	% of total in fruit	% of total in leaves, etc.	% of total in fruit	% of total in leaves, etc.	% of total in fruit	% of total in leaves, etc.
K 2	29·2	70·8	52·3	47·7	52·4	47·6
K 3	40·9	59·1	55·7	44·3	39·2	60·8

In both cases there is more phosphoric acid in the fruit. On the other hand, more potash is contained in the vegetative parts and the difference between the two cases is large. Whereas in K 2 (unmanured) there is almost two and a half times as much potash in the green parts as in the fruit, in K 3 the difference, although in the same direction, amounts to less than 50 per cent.

The nitrogen in the K 2 plants is distributed approximately equally between the green parts and the fruit, but in the K 3 plants about 50 per cent. more is present in the leaves, etc. than in the fruit.

Thus, manuring affects not only the quality and the quantity of the crop and the composition of the whole plant, but it also alters the distribution of the nitrogen, phosphoric acid and potash between the fruit and the vegetative parts of the plant.

The results for 1926.

The detailed results of the analyses of plants grown in house M in 1926 are shown in Tables V to IX. In considering these results it must be remembered that, when reference is made to growth or total nutrients per plant, the growth and contents of the plants at the time of planting have been deducted.

The rate of growth.

The average values for green weight and dry weight in Table V are plotted in Fig. 1. As the plants were not planted till March 5 they have all grown for four days less than the actual month by which they are designated. The curves show that most growth was made by plants in Group 5, *i.e.* those which had grown for five months only. Unfortunately, from the experimental point of view, the plants in this group were abnormally good. It is general experience in tomato culture that, despite care in the selection of seed and of uniformity in cultural operations, individual plants, and sometimes groups of two or three plants, will

exhibit marked differences from their immediate neighbours. The plants in Group 5 were in this category in spite of the fact that they were some feet away from one another, and they were all good plants. If the values for this group be ignored it will be seen that the curves showing

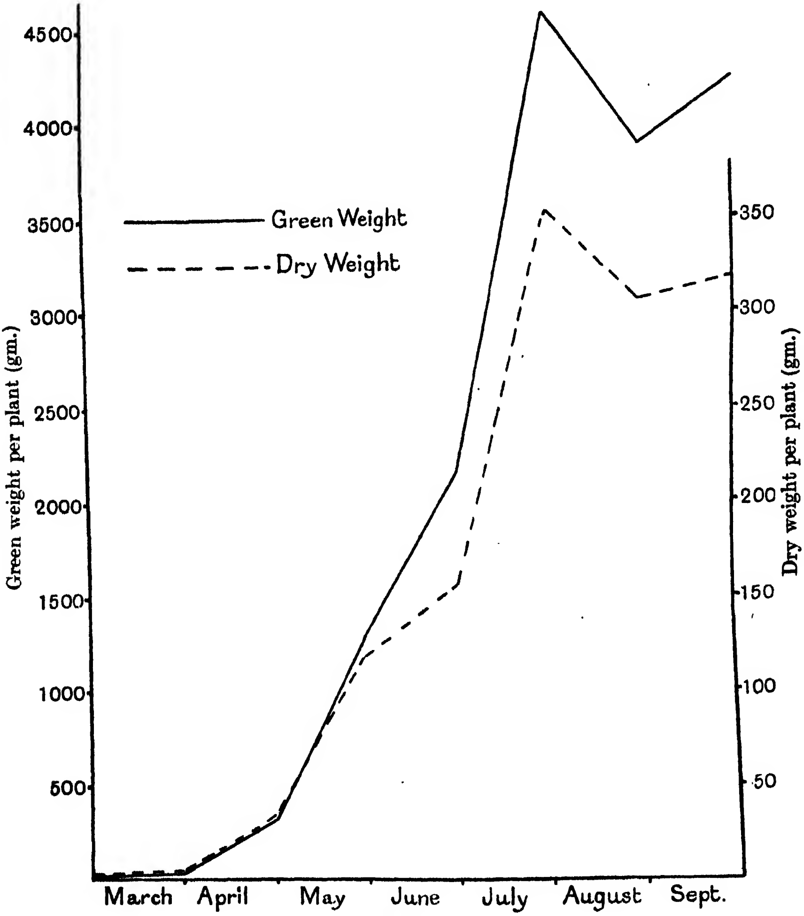

Fig. 1.

the rate of growth are similar to those usually obtained in this type of work. It will be seen also that a decided increase in the rate of growth occurs during May and another increase in the rate is experienced some time between the end of June and the end of August. Both these increases can be associated with the usual cultural operations. The plants were

Table V. *The total weights, in gm., of plants in house* M—1926.

Plant	Period of growth (mths.)	Green weight: Green parts	Green weight: Fruit	Green weight: Total	Dry weight: Green parts	Dry weight: Fruit	Dry weight: Total
At planting		7·7	—	7·7	0·6996	—	0·6996
1 *a*	1	43·5	—	43·5	4·397	—	4·397
1 *b*	1	50	—	50	4·357	—	4·357
1 *c*	1	40·8	—	40·8	3·958	—	3·958
2 *a*	2	417·2	—	417·2	33·48	—	33·48
2 *b*	2	249·3	—	249·3	29·54	—	29·54
2 *c*	2	310·7	—	310·7	40·23	—	40·23
3 *a*	3	575·5	414	989·5	59·05	25·27	84·32
3 *b*	3	633	320	953	105·4	23·12	128·52
3 *c*	3	956·9	942	1898·9	86·25	53·45	139·7
4 *a*	4	940	1533	2473	89·54	71·45	160·99
4 *b*	4	826	875	1701	81·68	57·67	139·35
4 *c*	4	912	1359	2271	96·63	68·18	164·81
5 *a*	5	1946·7	2056·4	4003·1	175·5	102·96	278·46
5 *b*	5	2325·2	2135	4460·2	305·5	123·26	428·76
5 *c*	5	1982·3	3251·5	5233·8	184·0	163·31	347·31
6 *a*	6	2310·5	1896·5	4207	252·7	92·52	345·22
6 *b*	6	2064·5	1944·1	4008·6	199·5	123·63	323·13
6 *c*	6	1240	2219·5	3459·5	138·8	109·42	248·22
7 *a*	7	1687·7	3331·8	5019·5	182·7	159·54	342·24
7 *b*	7	1273·5	3115·4	4388·9	140·2	158·89	299·09
7 *c*	7	2568	1779	4347	237·5	89·24	326·74
8 *a*	7	966·5	2466·8	3433·3	157·7	141·74	299·44
8 *b*	7	1600	3234·6	4834·6	199·6	147·31	346·91
8 *c*	7	1335·5	1930·6	3266·1	158·0	100·48	258·48

watered properly (as distinct from overhead damping on sunny days in March and April) for the first time on May 14 and the first top-dressing of the season was applied on May 21. The first watering always causes the plants to make quick growth and this is reflected in the value for May. The second increase in the rate of growth is probably due to a different cause. It is usual to "stop" the plants at the fifth truss, *i.e.* the growing point immediately above that truss is pinched out and a side-shoot below the truss is allowed to grow on. This encourages the fruit on the lower trusses to swell and ensures setting of the fruit on the sixth truss. This operation had been performed on practically all the plants in Groups 4 to 7 by the end of May. In quite a short time swelling of the fruit on the first two trusses was general. By the middle of June fruit had set on the first four trusses of all the plants. "Stopping" and the encouragement of ripening probably check vegetative development almost entirely for a short time and it is only after some of the fruit has been picked that the side-shoot makes much progress. This check probably accounts for the low value for June. During July and August the side-shoot makes normal development and the "top" of the plant generally

is growing. This increased production of leaves, etc. is responsible for the higher values in July and August. The state of affairs at the top of the plant does not lend itself to discussion. It is not customary to trim these parts as severely as the lower parts, but the effect of this on the September growth cannot be estimated since the houses were not heated and, in any case, in a normal year growth is not vigorous at this period.

Discussion of the results.

Tables VI to IX show the analytical data for all the plants. The figures for the ash values in Table VI show that the ash content of the dried green parts is almost twice that of the dried fruit. It also appears that the abnormality of the plants in Group 5 is associated with a high ash content.

Table VI. *The percentage ash in the dry matter of the fruit and green parts of plants in house* M—1926.

	Truss									
Plant	1	2	3	4	5	6	7	8	9	Green parts
1	—	—	—	—	—	—	—	—	—	22·46
2 *a*	—	—	—	—	—	—	—	—	—	20·69
2 *b*	—	—	—	—	—	—	—	—	—	21·05
2 *c*	—	—	—	—	—	—	—	—	—	22·21
3 *a*	11·58	12·23	—	—	—	—	—	—	—	21·30
3 *b*	10·20	10·71	—	—	—	—	—	—	—	20·39
3 *c*	12·00	13·69	12·51	—	—	—	—	—	—	20·36
4 *a*	13·26	13·03	13·02	11·54	—	—	—	—	—	24·82
4 *b*	11·42	12·25	11·72	11·83	—	—	—	—	—	25·87
4 *c*	10·94	12·42	12·16	12·24	—	—	—	—	—	21·56
5 *a*	11·02	11·39	13·05	13·86	11·51	12·39	—	—	—	26·28
5 *b*	13·10	11·76	14·21	14·11	13·24	12·53	12·01	—	—	25·27
5 *c*	13·37	13·27	12·86	12·72	14·85	—	—	—	—	23·29
6 *a*	13·31	13·24	15·09	16·01	13·27	14·55	—	—	—	24·32
6 *b*	13·58	12·77	14·44	15·21	14·19	12·43	10·51	—	—	22·92
6 *c*	14·59	12·50	13·55	14·20	15·29	13·41	—	—	—	24·27
7 *a*	12·25	12·80	11·77	13·83	12·45	10·31	11·02	10·68	10·44	24·39
7 *b*	13·14	16·31	12·43	12·55	10·01	12·34	—	—	—	25·18
7 *c*	11·49	12·11	13·86	14·21	14·14	11·01	13·05	—	—	26·08
8 *a*	10·39	10·94	13·80	14·64	13·94	13·04	13·02	12·10	—	22·58
8 *b*	11·37	12·24	14·43	12·23	11·13	11·47	11·76	11·15	—	20·05
8 *c*	11·18	10·66	13·50	13·01	12·59	12·52	12·85	13·55	—	22·31

Composition of trusses on different parts of the plants.

The tables (VII, VIII, IX) showing the composition of the plants offer several points of interest. The fruit on the plants in Groups 6, 7 and 8 was practically all ripe in the commercial sense and Fig. 2 gives the average values for such fruit. The differences in composition are not great but the curves suggest that for all three constituents there is an

Table VII. *The percentage nitrogen in the dry matter of the fruit and green parts of plants in house* M—1926.

Plant	Truss 1	2	3	4	5	6	7	8	9	Green parts
1	—	—	—	—	—	—	—	—	—	3·649
2 *a*	—	—	—	—	—	—	—	—	—	3·518
2 *b*	—	—	—	—	—	—	—	—	—	3·392
2 *c*	—	—	—	—	—	—	—	—	—	3·237
3 *a*	3·43	3·20	—	—	—	—	—	—	—	2·847
3 *b*	2·47	3·28	—	—	—	—	—	—	—	3·118
3 *c*	3·26	3·39	3·08	—	—	—	—	—	—	2·710
4 *a*	2·77	2·94	2·31	3·45	—	—	—	—	—	3·172
4 *b*	2·68	2·89	2·75	2·94	—	—	—	—	—	4·171
4 *c*	2·51	3·57	3·19	2·66	—	—	—	—	—	3·047
5 *a*	2·88	3·40	3·95	3·46	3·72	3·70	—	—	—	3·074
5 *b*	3·85	2·84	3·88	3·54	3·83	3·55	4·04	—	—	3·036
5 *c*	3·42	2·66	3·16	3·30	3·71	—	—	—	—	2·903
6 *a*	2·99	3·25	3·72	3·64	3·40	3·49	—	—	—	3·102
6 *b*	3·10	2·62	3·94	3·51	3·24	3·77	3·21	—	—	3·088
6 *c*	3·27	3·06	3·18	3·44	3·55	3·64	—	—	—	3·138
7 *a*	3·29	2·71	3·07	3·21	3·05	2·74	2·63	2·69	2·94	2·688
7 *b*	3·27	3·21	3·06	2·85	2·90	3·49	—	—	—	2·902
7 *c*	3·67	4·10	3·41	4·14	4·11	3·24	4·20	—	—	2·923
8 *a*	2·75	3·48	3·52	3·25	3·64	3·41	3·45	3·57	—	2·910
8 *b*	2·98	3·84	3·52	3·13	3·05	2·93	2·66	3·04	—	2·636
8 *c*	2·58	2·52	3·56	3·52	4·24	4·02	3·44	3·62	—	2·769

Table VIII. *The percentage potash in the dry matter of the fruit and green parts of plants in house* M—1926.

Plant	Truss 1	2	3	4	5	6	7	8	9	Green parts
1	—	—	—	—	—	—	—	—	—	7·91
2 *a*	—	—	—	—	—	—	—	—	—	6·36
2 *b*	—	—	—	—	—	—	—	—	—	6·40
2 *c*	—	—	—	—	—	—	—	—	—	7·45
3 *a*	6·06	7·17	—	—	—	—	—	—	—	6·71
3 *b*	8·70	6·08	—	—	—	—	—	—	—	5·81
3 *c*	5·29	7·80	8·65	—	—	—	—	—	—	7·35
4 *a*	6·35	7·82	5·80	6·39	—	—	—	—	—	5·67
4 *b*	5·62	6·46	6·13	5·89	—	—	—	—	—	7·44
4 *c*	5·07	6·33	7·37	6·73	—	—	—	—	—	5·53
5 *a*	6·31	5·97	6·50	6·92	6·19	7·25	—	—	—	6·65
5 *b*	7·89	6·63	7·45	7·48	6·94	6·19	6·42	—	—	6·17
5 *c*	7·42	7·05	6·72	7·13	7·85	—	—	—	—	6·30
6 *a*	6·17	7·89	7·98	6·14	5·51	6·14	—	—	—	6·19
6 *b*	6·18	6·19	8·06	8·63	6·16	6·35	5·64	—	—	5·19
6 *c*	6·98	5·68	7·61	5·29	5·46	6·04	—	—	—	6·76
7 *a*	6·51	5·74	5·79	8·26	5·49	5·70	5·98	5·76	5·87	5·65
7 *b*	7·47	8·98	5·65	6·14	6·92	6·98	—	—	—	5·96
7 *c*	5·51	6·42	7·87	7·59	7·82	7·40	7·55	—	—	6·69
8 *a*	5·43	6·41	7·70	8·19	6·27	7·01	7·52	6·80	—	5·44
8 *b*	5·95	6·92	7·14	6·26	5·62	6·14	5·61	6·85	—	5·78
8 *c*	5·72	5·20	7·99	7·01	5·94	6·14	6·95	7·04	—	5·62

Table IX. *The percentage phosphoric acid in the dry matter of the fruit and green parts in house* M—1926.

Plant	Truss 1	2	3	4	5	6	7	8	9	Green parts
1	—	—	—	—	—	—	—	—	—	0·746
2 *a*	—	—	—	—	—	—	—	—	—	0·586
2 *b*	—	—	—	—	—	—	—	—	—	0·721
2 *c*	—	—	—	—	—	—	—	—	—	0·710
3 *a*	0·66	0·93	—	—	—	—	—	—	—	0·574
3 *b*	0·81	0·98	—	—	—	—	—	—	—	0·516
3 *c*	0·59	0·99	1·08	—	—	—	—	—	—	0·646
4 *a*	0·66	0·78	0·76	0·69	—	—	—	—	—	0·435
4 *b*	0·78	0·79	0·87	0·86	—	—	—	—	—	0·612
4 *c*	0·93	0·84	0·98	0·96	—	—	—	—	—	0·604
5 *a*	0·79	0·80	0·74	1·19	0·85	0·92	—	—	—	0·746
5 *b*	1·27	0·72	0·89	1·22	0·98	1·08	1·00	—	—	0·746
5 *c*	0·95	0·85	0·83	0·87	1·04	—	—	—	—	0·585
6 *a*	0·85	1·04	1·25	0·86	1·09	0·88	—	—	—	0·857
6 *b*	1·07	0·88	1·30	1·24	0·89	1·10	0·96	—	—	0·719
6 *c*	1·00	0·79	0·86	0·75	0·77	0·67	—	—	—	0·578
7 *a*	0·86	0·90	0·93	1·10	0·88	0·75	0·63	0·66	0·82	0·512
7 *b*	1·08	0·83	0·79	0·87	0·95	0·82	—	—	—	0·564
7 *c*	0·74	0·96	1·09	1·02	1·08	0·81	1·07	—	—	0·636
8 *a*	0·73	0·78	0·90	0·92	0·84	0·82	0·72	0·72	—	0·577
8 *b*	0·75	1·04	1·40	0·92	0·80	0·74	0·73	0·82	—	0·690
8 *c*	0·86	0·54	0·97	0·97	0·87	0·77	0·93	0·71	—	0·587

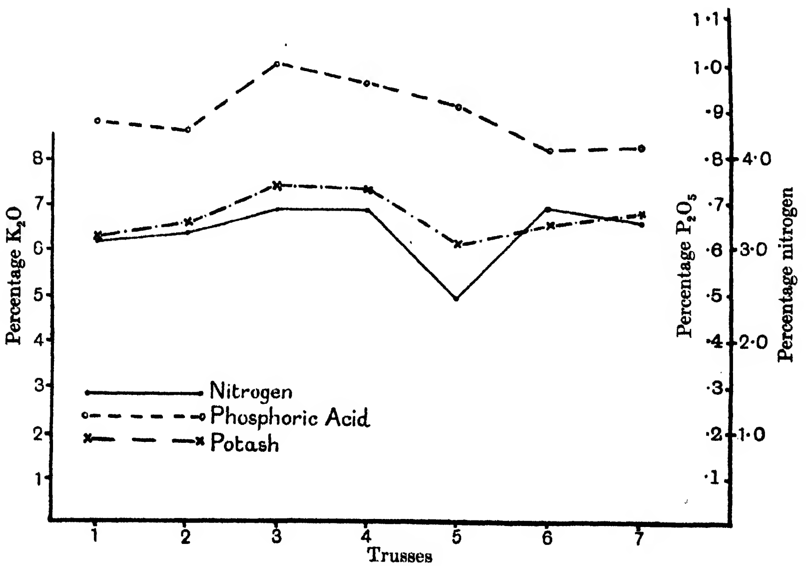

Fig. 2.

increase from the first to the third truss which with the fourth contain the maximum percentages. In the trusses above these the nitrogen content fluctuates somewhat irregularly. The potash value falls in the fifth truss, then increases in the sixth and seventh, while the phosphoric acid decreases gradually from the third truss upwards.

The effect of ripening on the composition of the fruit.

The fruit on the plants in Groups 3 and 4, generally, was not ripe. None of the fruit in Group 3 was ripe but that on the lowest truss was the least unripe. The fruit from the Group 4 plants was in a more mature condition and on the first truss one or two fruits had begun to colour. The average figures per truss are as follows:

		Nitrogen		
Truss:	1	2	3	4
Group 3	3·05	3·29	3·08	—
„ 4	2·65	3·13	2·75	3·02
		Potash		
Truss:	1	2	3	4
Group 3	6·68	7·02	8·65	—
„ 4	5·68	6·87	6·43	6·34
		Phosphoric acid		
Truss:	1	2	3	4
Group 3	0·69	0·97	1·08	—
„ 4	0·79	0·80	0·87	0·84

Without exception the first truss has the lowest percentage content of nutrients. If the differences are significant they suggest that the concentrations fall as the fruit ripens, in other words the intake of the three nutrients to the fruit is a slower process than the absorption of water and the elaboration of organic compounds.

The total removal of nutrients from the soil by the whole plants.

Table X gives these quantities. The figures show that as for the plants analysed in 1925, the phosphoric acid removed from the soil by the aerial parts of the plant is considerably lower in amount than are the nitrogen and potash. Table X shows that the *relative* potash content of the plants falls as the season progresses. Nursery experience is that plants generally appear to lack potash in the early part of the season. These figures may mean that the potash requirements are more easily met in the later part of the growth period. There is also a slight fall in the relative nitrogen values. Here the explanation is probably different since tomato plants generally show symptoms of a deficiency at the

end of the season and the decline here noted may indicate the difficulty of the plants in obtaining nitrogen.

Table X. *The average contents of the plants (in gm.) and the ratio* $K_2O : P_2O_5 : N$ *in each month.*

Months of growth	K_2O	P_2O_5	N	Ratios K_2O	P_2O_5	N
1	0·335	0·032	0·154	10·47	1	4·81
2	2·338	0·232	1·163	10·08	1	5·01
3	7·733	0·762	3·654	10·15	1	4·80
4	10·197	1·091	4·685	9·35	1	4·29
5	22·843	2·757	11·682	8·29	1	4·30
6	19·149	2·510	10·184	7·63	1	4·06
7	19·803	2·248	9·285	8·81	1	4·12

Conclusions.

Considered in conjunction with the results of manurial trials the data presented here show that the needs of the tomato plant for phosphates as a direct nutrient are low. It is possible however, that the absence of phosphates reduces the intake of potash. Consequently, plants receiving little or no phosphates may yield good crops but the potash content of such plants will be low. This may or may not affect the flavour of the fruit. It must be remembered that in all the work the only source of potash has been the sulphate and it is possible that different results might follow the use of some other salt. This is a point which is to receive attention.

The analyses of manured and unmanured plants draw attention to the interesting problem of the tomato plants as a fruit-producing system. The data show that on a basis of fruit produced per unit weight of green matter the unmanured plants appeared to be the more efficient. Actually this is not the case, since the fruit from the unmanured plot was of inferior quality and showed a low potash content. The weight of fruit per plant was also low.

Another point of interest arises from a comparison of the distribution of the nutrients between the fruit and the remainder of the aerial parts of the plants in the two cases. Manuring increases the proportion of phosphoric acid and potash in the fruit but depresses the proportion of nitrogen. This and the analytical data for the fruit in the two cases suggest that inferior quality fruit produced by the unmanured plants contains less potash and phosphoric acid but more nitrogen than good fruit produced by plants receiving adequate manurial treatment.

The effects of cultural operations such as watering and "stopping" are reflected in the rate of growth of the plant. The first watering is followed by a decided increase in the rate of growth. "Stopping," which checks growth temporarily, causes a depression which is followed by an increase in the rate when new growth is making headway.

A comparison of the trusses on manured plants suggests that the third and fourth trusses are the richest in the three nutrients in respect of which analyses were made. This is of interest in view of the fact that there are indications that the seed from these trusses is highly satisfactory from the commercial standpoint.

SUMMARY.

In an attempt to determine the rôle of phosphates in tomato culture tomato plants have been analysed for potash, phosphoric acid and nitrogen.

In unmanured plants the fruit is of inferior quality but the weight as picked is 2·6 times that of the foliage and stems of the same plants. In manured plants this ratio is 1·6. The relative efficiency of the plants under the two sets of conditions is discussed.

Manured plants have a higher ash content than unmanured plants.

The removal of the three nutrients per plant is:

	K_2O	P_2O_5	N
Manured (gm.)	18·22	2·028	9·324
Unmanured (gm.)	2·775	0·9895	4·922

Analyses of trimmings and leaves at different times of the year suggest that the actively growing parts are richest in the three nutrients.

The composition of fresh fruit is as follows:

	H_2O	K_2O	P_2O_5	N
Manured plants (%)	93·5	0·3237	0·0491	0·1591
Unmanured plants (%)	94·9	0·0556	0·0355	0·1769

The distribution of the total nutrients contained in the plants between the fruit and the vegetative parts is different for manured and unmanured plants.

The rate of growth of the tomato plant in a well-manured soil is irregular, being influenced by cultural operations.

Tomato plants yielding good crops show a relatively high ash content.

Of all trusses on a plant the third and fourth are richest in the three nutrients determined. The effect of ripening on the composition of the fruit is discussed.

The relative amount of potash in the whole of the aerial parts of the plant tends to fall as the season progresses.

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PYRETHRIN I AND II.

THEIR ESTIMATION IN PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM*). II.

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CONTENTS.

Introduction.

We have recently described two chemical methods for the determination of the poisons in the insecticidal plant pyrethrum (*C. cinerariaefolium*), based on the researches of Staudinger and his co-workers whose results we were able to confirm. Working with pyrethrum grown in England from Swiss seed, we obtained results which could be correlated closely with the observed toxicity of the samples and of the pure poisons themselves.

Staudinger and Ruzicka (3) isolated from pyrethrum of European origin two active principles, named by them pyrethrin I and II, to which they ascribe the whole of the insecticidal action and whose structures they determined. The Japanese workers Fujitani (2) and Yamomoto (6), however, arrived at different conclusions as to the nature of the active principles and this has led to the suggestion (4) that different poisons may be produced by the same plant, *Chrysanthemum cinerariaefolium*, grown in Japan and Europe. Although this hypothesis is highly improbable, it nevertheless seemed important to establish the validity of the analytical process, not only for European pyrethrum, but also for pyrethrum grown from Japanese seed, as in the event of failure it would not be possible to propose the method as a general one.

Of the two methods already mentioned, the acid method is the more convenient but it is nevertheless a somewhat lengthy process, taking several days to complete. As it had been shown that of the two poisons, pyrethrin I is mainly responsible for the toxicity, it was decided to

explore the possibility of making a rapid evaluation of pyrethrum by estimating pyrethrin I alone by an abbreviated method.

This paper deals with the results of the evaluation, by both chemical and biological means, of pyrethrum grown from Swiss and Japanese seed and includes in addition a study of a rapid approximate method for the chemical assay of pyrethrin I.

Comparison of pyrethrum grown from Swiss and Japanese seed.

During 1928 a considerable number of tests was carried out on the toxicities of the flowers derived from Swiss seed and grown on plots laid down at stations in different parts of the country. Unfortunately, however, towards the latter part of the summer of that year a shortage of suitable insects, particularly *Aphis rumicis*, made it impossible to test adequately the samples grown from Japanese seed. Samples grown in 1926 from Swiss and Japanese seed had been tested in the previous year (1927) in a number of toxicity trials, full particulars of which are published elsewhere (1), and as these samples were still available we have determined in them the content of pyrethrin I and II, by means of the acid method previously described (5). The data are given in Table I.

Table I. *Analyses of Pyrethrum Flowers.*

Station and derivation of seed	Full acid method: Pyrethrin I %	Full acid method: Pyrethrin II %	Short acid method: Pyrethrin I %	Toxicities to *A. rumicis*: Highest conc. giving *under* 50 % moribund and dead. Gm. flowers/100 c.c.	Toxicities to *A. rumicis*: Lowest conc. giving *over* 50 % moribund and dead. Gm. flowers/100 c.c.
Crop 1926					
Scillies. Swiss	0·59	0·53	0·60	—	0·025 (60 %)*
Wye. Swiss	0·36	0·32	—	0·05 (20 %)	0·1 (75 %)
Swanley. Japan.	0·30	0·44	0·33	0·05 (10 %)	0·1 (80 %)
Harpenden. Japan.	0·29	0·50	0·27	0·05 (30 %)	0·1 (70 %)
Harpenden. Swiss	0·28	0·34	—	0·05 (20 %)	0·1 (75 %)
Sparsholt. Japan.	0·26	0·28	0·27	0·05 (20 %)	0·1 (70 %)
E. Malling. Japan.	0·20	0·24	0·19	0·1 (30 %)	0·25 (100 %)
Wye. Japan.	0·18	0·30	0·15	0·1 (30 %)	0·25 (100 %)
Crop 1927					
Worcester. Swiss	0·45	—	0·43	—	—
Scillies. Swiss	0·44	—	0·43	—	—
Swanley. Swiss	0·41	—	0·38	—	—
Wisley. Swiss	0·39	—	0·35	—	—
Wye. Swiss	0·36	—	0·36	—	—
Seale Hayne. Swiss	0·28	—	0·26	—	—

* The figures in brackets indicate the observed mortalities at the concentrations given.

For purposes of comparison of the analytical results with the toxicity data, we have in the table represented briefly the insecticidal values of

the different samples in the following manner. The samples were tested ((1), p. 430) at concentrations, in terms of flowers, of 0·5, 0·25, 0·1, 0·05, and 0·025 gm. per 100 c.c. of spray fluid. Statistically, the concentrations giving 50 per cent. of moribund and dead insects are the most suitable for purposes of comparison, but in view of the difficulties of interpolation, we have stated the highest of the concentrations tested giving *below* 50 per cent. and the lowest giving *over* 50 per cent. of moribund and dead insects.

A consideration of the table shows that for the 1926 crop the samples can be arranged in three groups according to their toxicities and that this grouping can be correlated with the content of pyrethrin I. Thus, the Scillies (Swiss) sample contains 0·59 per cent. and is significantly more toxic than the others, while in the second group of medium toxicity is included a number of samples, derived from both Japanese and Swiss seed, and containing from 0·26 to 0·36 per cent. of pyrethrin I. In the third and least toxic group are placed the two lots of flowers grown from Japanese seed at East Malling and Wye, and containing 0·20 and 0·18 per cent., respectively, of pyrethrin I. In addition to the difficulty of reducing toxicity data to an expression by two figures, the above method of representation can only be regarded as approximate owing to the wide separation of concentrations tested; but it should be stated that a more complete examination of the full data, given by Fryer, Tattersfield and Gimingham ((1), p. 430), gives general confirmation for the deductions drawn. It is, therefore, hardly a matter of doubt that the acid method is as suitable for testing flowers from Japanese seed as it is for those grown from Swiss seed, and it seems almost certain that the poisons are identical in the two cases.

SHORT METHOD FOR DETERMINING PYRETHRIN I.

The acid method of analysis takes several days to complete and the number of operations required is somewhat large. Seeing that pyrethrin I has been shown to be much the more important poison of the two (5), it was considered advisable to attempt to devise a more expeditious method for its determination. Even if this method were only approximately correct, it would be of great use in sieving out good from poor samples and so materially lessening the labour of differentiating between a large number of different specimens. The longer method could then be limited to the analysis of this smaller group. The following method has accordingly been applied to a number of samples previously analysed by the longer method.

10 gm. of the ground pyrethrum are extracted in a Soxhlet apparatus by means of petroleum ether (boiling range 40 to 50° C.), which is kept vigorously boiling over a carbon-filament lamp. The extraction is continued until the ether draining over is colourless. The petroleum ether solution which should have a volume of approximately 50 c.c. is then poured into a long-necked flask of 100 c.c. capacity, subsequently used for distillation, the extraction flask being rinsed once with a little petroleum ether, 4 to 5 c.c. of $N/1$ caustic soda in methyl alcohol added and the mixture vigorously refluxed on the water-bath for 1½–2 hours. The mixture is then acidified with $N/1$ sulphuric acid and distilled in steam in the apparatus previously described (5). Petroleum ether distils first and until it is completely removed, a flame is not placed under the distillation flask. To prevent risk of fire the worm-condenser is attached to the receiver by means of a cork with two holes, one of which contains a glass tube with a length of indiarubber tubing to carry any inflammable vapour away from the flame. Distillation is continued until 50 c.c. of aqueous distillate stand below the petroleum ether in the receiver, after which another flask is attached and distillation continued until a further 50 c.c. have distilled. The whole of the first distillate is then transferred to a fairly large separating-funnel and vigorously shaken for one minute, the aqueous layer is separated and the petroleum ether layer, after washing once with water, is run off into a flask containing 20 c.c. of water, to which a few drops of alcohol and phenolphthalein have been previously added together with just enough alkali to render the liquid a faint pink. Titration is carried out with $N/50$ soda until the aqueous layer is distinctly alkaline after vigorous shaking in the corked flask. The second 50 c.c. of distillate is added to the first aqueous fraction (which has already been extracted once), vigorously shaken in a separating-funnel with 50 c.c. of petroleum ether, the washed petroleum ether layer added to the titration flask and the titration finished as before; very little additional $N/50$ soda is usually required. After deducting a blank which should be determined for the petroleum ether (about 0·2 c.c. $N/50$ soda), the monocarboxylic acid and pyrethrin I content can be calculated, the following factors being used:

$$1 \text{ c.c. } N/50 \text{ alkali} = \tfrac{168}{50} = 3{\cdot}36 \text{ mg. monocarboxylic acid,}$$

$$\text{,,} \qquad = \tfrac{330}{50} = 6{\cdot}6 \text{ mg. pyrethrin I.}$$

Table I includes data obtained by this method and indicates how close are the agreements with the figures obtained by the longer process. Thus, while the method gives results of sufficient accuracy for most

purposes, it can be carried out in a few hours and on a small amount of material. So far, however, we have not found it possible to determine the water-soluble acid in the residue from the distillation, but as pyrethrin II is less toxic than pyrethrin I, this is not a serious omission.

SUMMARY.

1. The acid method previously described has been used to evaluate samples of pyrethrum derived from both Swiss and Japanese seed, with equally successful results.

2. A rapid method for the evaluation of pyrethrum by a determination of pyrethrin I is described.

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NORMAL DAY TO DAY VARIABILITY OF YIELD OF MILK AND FAT OF INDIVIDUAL COWS.

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Introduction.

Records of the quantity of milk yielded by cows are comparatively easy and inexpensive to obtain, but records of quality of milk involve a much greater expense and for this reason are often neglected. It is the usual practice when recording the fat percentage of the milk of individual cows to test the milk of each cow for a few days only during each lactation period and to estimate the true quality of the milk from these few days' records. It will be obvious that the result obtained will not be accurate and the question arises as to how near the truth will be a record based on a given number of tests or, alternatively, what is the smallest number of tests necessary in order to produce a result within certain limits of accuracy.

One of the chief reasons why a small number of tests for butterfat is inaccurate for deciding the true production of a cow is that for no obvious reason the fat percentage of every cow varies considerably from day to day, so that the result of a single test is almost invariably slightly above or below the true average; if several tests are taken, however, the ordinary laws of chance decide that a proportion of the tests will yield results which are too high and a proportion too low so that an average of a number of such results will tend to approximate to the truth. By the application of certain methods and formulae well known to mathematicians it is possible to forecast and to express the probable degree of accuracy likely to be obtained under given conditions, and it was with these facts in mind that the work discussed in this paper was undertaken.

Other problems connected with the variability of milk and butterfat have arisen during the course of the work and brief reference has been made to certain aspects of the present law governing the quality of milk for sale in this country and to the secretion of milk.

MATERIAL AND METHODS.

Two sets of data were used in this work:

(1) Three cows had been used as controls in experimental work at the Institute and records were available of the weight of milk and the butterfat content of every milking throughout a lactation period. The records of these cows were divided into sections of 21 days, the first section commencing about 6 days after calving when the yields had become reasonably uniform. The standard deviations of the fat percentages were calculated for each section of 21 days, the morning and evening milkings being treated separately.

From the results obtained it was noted that daily fluctuations in fat percentages vary with the stage of lactation and with the season of the year and it was decided to consider day to day variability as affected by these two factors. Also it was decided to deal with weights of milk and fat yielded at each milking as well as the fat percentages of milk.

While the data from these three cows were of value for certain aspects of this work, they were unsuitable for the main study to be undertaken because of the fact that all three cows calved in the autumn.

(2) The other set of data employed for this work were monthly records of individual animals in a herd sampled at each milking on three consecutive days each month; the records covered five years and some details as to management of the herd have been noted in a previous paper(1), but since the day to day variations in the yield of cows are affected by many points of management, more particularly by changing or inefficient milkers, by variable times of milking and rough treatment of the cows, it seems desirable to add some further notes regarding the herd management of the cows under discussion.

Only efficient milkers were employed and each man milked the same cows day after day, changes taking place only during the week-ends owing to half-day holidays, and when sickness necessitated the employment of fresh milkers. With regard to times of milking, it was exceptional for the time of milking any cow to vary more than 10 minutes from one day to another except on Sundays, thus the only avoidable disturbance occurred during the week-ends. As the samples for butterfat were taken on a Tuesday, Wednesday and Thursday about the middle of each calendar month, the influence of these week-end disturbances should not be evident on the recorded days. The cows were treated quietly, having been accustomed to regular handling from birth onwards, and they did not appear to be disturbed by the daily quota of visitors.

It is believed that the day to day fluctuations in these records are as low as is possible under practical conditions and there is little doubt that on most farms the variability is considerably greater, particularly during the week-ends.

In the treatment of this material the morning and evening yields were taken separately, and the standard deviations were calculated by taking the mean of three consecutive morning (or evening) records from one cow and finding the deviation of each of the three variates from this mean. By repeating the process for the record of each cow in the group under consideration, sufficient variates were obtained to give a satisfactory result. The sum of the squares of the deviations so obtained provided the necessary material for calculating the standard deviations.

Comparison between the day to day variability of different cows.

Table I presents a summarised statement of the results obtained from the three cows which were sampled and tested for fat at every milking throughout a lactation period. The table will be referred to later, but under the above heading it is only necessary to draw attention to points in which the table helps to indicate the comparative variability of fat percentage in different cows.

In order to test this point with these three cows when under almost identical conditions, the seven periods of three weeks from the 5th to the 25th week after calving were compared. During this period the cows were neither disturbed by "after calving" effects nor by summer grazing conditions, and the mean values shown in the last column of Table I will assist in this comparison. Using the coefficient of variability as a basis, it will be found that "Bertha" at morning milkings gave a mean figure of 7·32, the maximum and minimum values for any single period being 12·5 and 3·2. The corresponding figures for the evening milkings were a mean of 8·36 with individual periods varying from 14·0 to 3·7. "Welcome's" morning figures were a mean of 8·74 with extremes of 18·9 and 4·7, the evening mean figure being 8·33 with extremes of 12·4 to 3·9. "Flora" gave similar results with a morning mean of 8·36 varying from 13·2 to 3·6 and evening mean of 8·47 varying from 12·1 to 6·2.

It will be seen from these results that the variability in the fat percentage of any single cow cannot be calculated with an approach at accuracy if the figures are based on a test period of 21 consecutive

days; on the other hand when seven of such periods were averaged a close agreement was found between the three cows.

If, as is most probable, the day to day variability of fat percentage is due partly to the milker and partly to the nervous condition of the cow, such a result is to be expected since some of the 21 day periods must coincide with changes in milkers or a condition of the cow when her health or nervous condition is slightly abnormal. The chief point which it is desired to make at this stage is that the inequalities which occur between the variability of the fat percentage of different cows tend to balance out when such cows are kept under similar conditions for a long period. It is admitted that the results from three cows do not provide conclusive evidence on this point.

DAY TO DAY VARIABILITY OF MILK AND FAT YIELDS AS AFFECTED BY STAGE OF LACTATION.

In order to reduce the work involved in the study of the above factor, only about half of the available records were utilised, viz. those of cows calving during alternate months, January, March, May, and so on. All the cows which calved in January were grouped together and standard deviations (S.D.) for milk and fat yields were calculated by the method already described and figures obtained for each of the first eight months of the lactation. By similar treatment of the March, May and other groups of cows, S.D. were calculated for a large number of cows calving at regular intervals throughout the year, and by preparing Table II from this set of results it is believed that the true influences of the stage of lactation have been found and that the influence of the season of the year has been eliminated. Table II is classified in three sections and shows the day to day variability of (*a*) milk yield, (*b*) yield of fat, and (*c*) fat percentages, in respect of morning and evening milkings during each of the first eight months of lactation. Dealing firstly with milk yields, Table II section (*a*), the mean yields per milking do not call for comment. The S.D. during the first month of lactation are considerably higher than during any other month and from the second to the eighth month the S.D. are fairly constant, also the morning and evening milkings yield S.D. which are closely comparable, *i.e.* 0·77 and 0·76 respectively. The coefficients of variability which were calculated by the usual formula (S.D. × 100/Mean) are high during the first month of lactation, followed by low figures for the month of maximum production and then tending toward a slight rise with the advance of lactation, *i.e.* after the first month the tendency is for the C.V. to vary

Table I. *Showing variability in the fat percentage of the milk of three*

No. of weeks after calving →		2 to 4	5 to	8 to 10	11 to 13	14 to 16	17 to 19
Bertha (second calf produced 30. x. 24)							
a.m. milkings	Mean % Fat	3·92±0·10	3·60±0·05	3·11±0·06	2·91±0·04	3·25±0·03	3·30±0·03
	S.D.	0·65±0·07	0·35±0·04	0·39±0·04	0·27±0·03	0·21±0·02	0·22±0·02
	C.V.	16·5 ±1·7	9·7 ±1·0	12·5 ±1·3	9·1 ±0·9	6·5 ±0·7	6·6 ±0·7
p.m. milkings	Mean % Fat	5·89±0·10	4·72±0·10	4·43±0·09	4·16±0·04	4·42±0·06	4·53±0·05
	S.D.	0·64±0·07	0·66±0·07	0·60±0·06	0·30±0·03	0·40±0·04	0·33±0·03
	C.V.	10·8 ±1·1	14·0 ±1·5	13·5 ±1·4	7·1 ±0·7	9·0 ±0·9	7·2 ±0·8
Welcome (second calf produced 27. x. 24)							
a.m. milkings	Mean % Fat	2·35±0·05	2·04±0·02	2·28±0·06	2·60±0·04	2·71±0·03	2·97±0·03
	S.D.	0·33±0·03	0·12±0·01	0·43±0·04	0·28±0·03	0·18±0·02	0·17±0·02
	C.V.	14·0 ±1·5	5·9 ±0·6	18·9 ±2·0	10·8 ±1·1	6·6 ±0·7	5·7 ±0·6
p.m. milkings	Mean % Fat	4·07±0·06	3·66±0·05	4·03±0·07	4·05±0·07	4·00±0·04	3·93±0·02
	S.D.	0·40±0·04	0·34±0·04	0·50±0·05	0·48±0·05	0·27±0·03	0·15±0·02
	C.V.	9·9 ±1·0	9·3 ±1·0	12·4 ±1·3	11·8 ±1·2	6·8 ±0·7	3·9 ±0·4
Flora (first calf produced 19. xi. 24)							
a.m. milkings	Mean % Fat	2·89±0·11	2·26±0·05	2·66±0·05	2·81±0·04	3·01±0·04	2·87±0·03
	S.D.	0·78±0·08	0·35±0·04	0·35±0·04	0·24±0·03	0·26±0·03	0·20±0·02
	C.V.	27·1 ±2·8	10·9 ±1·1	13·2 ±1·4	8·5 ±0·9	8·5 ±0·9	6·8 ±0·7
p.m. milkings	Mean % Fat	5·58±0·13	4·20±0·08	4·13±0·05	3·91±0·04	3·47±0·05	3·47±0·03
	S.D.	0·91±0·09	0·51±0·05	0·35±0·04	0·28±0·03	0·32±0·03	0·22±0·02
	C.V.	16·3 ±1·7	12·1 ±1·3	8·5 ±0·9	7·1 ±0·7	9·2 ±1·0	6·2 ±0·6
Mean values for the a.m. and p.m. milkings of three cows							
	Mean % Fat	4·12	3·41	3·44	3·41	3·48	3·51
	S.D.	0·62	0·39	0·44	0·31	0·27	0·22
	C.V.	15·8	10·3	13·2	9·1	7·8	6·1

Table III. *Showing the comparative variability of the milk and*

		Jan.	Feb.	March	April	May	June
(*a*) **Milk yield**							
a.m. milkings	Mean (lb.)	13·7 ±·07	14·1 ±·03	13·9 ±·03	15·3 ±·04	15·1 ±·07	15·5 ±·04
	S.D.	1·01 ±·05	0·48 ±·02	0·47 ±·02	0·64 ±·03	1·19 ±·05	0·68 ±·03
	C.V.	7·7 ±·37 (5·6)†	3·6 ±·16	3·8 ±·16	4·4 ±·17	7·8 ±·31	4·5 ±·18
p.m. milkings	Mean (lb.)	8·3 ±·04	9·1 ±·06	8·5 ±·05	9·5 ±·05	10·5 ±·06	10·6 ±·06
	S.D.	0·65 ±·03	0·46 ±·02	0·42 ±·02	0·57 ±·02	1·25 ±·05	0·62 ±·03
	C.V.	8·4 ±·40 (5·4)†	5·4 ±·23	6·5 ±·28	6·0 ±·24	10·8 ±·46	6·0 ±·24
Mean values for the a.m. and p.m. milkings							
	Mean (lb.)	11·0 ±·06	11·6 ±·07	11·2 ±·07	12·4 ±·07	12·8 ±·07	13·0 ±·07
	S.D.	0·83 ±·04	0·47 ±·02	0·44 ±·02	0·61 ±·02	1·22 ±·05	0·65 ±·03
	C.V.	8·0 ±·38 (5·5)†	4·5 ±·19	5·2 ±·22	5·2 ±·21	9·3 ±·37	5·3 ±·22
(*b*) **Yield of fat**							
a.m. milkings	Mean (lb.)	0·48 ±·004	0·49 ±·002	0·53 ±·002	0·51 ±·003	0·49 ±·003	0·50 ±·003
	S.D.	0·063 ±·003	0·035 ±·002	0·037 ±·002	0·045 ±·002	0·061 ±·002	0·053 ±·002
	C.V.	13·2 ±·64 (9·0)†	7·4 ±·32	6·8 ±·29	8·9 ±·35	12·5 ±·50	10·5 ±·43
p.m. milkings	Mean (lb.)	0·38 ±·004	0·42 ±·003	0·41 ±·002	0·40 ±·002	0·45 ±·004	0·46 ±·003
	S.D.	0·054 ±·003	0·045 ±·002	0·038 ±·002	0·039 ±·002	0·066 ±·003	0·058 ±·002
	C.V.	14·6 ±·70 (10·6)†	11·1 ±·48	9·3 ±·40	9·6 ±·38	14·5 ±·58	12·6 ±·51
Mean values for the a.m. and p.m. milkings							
	Mean (lb.)	0·43 ±·004	0·46 ±·002	0·47 ±·002	0·46 ±·002	0·47 ±·004	0·48 ±·003
	S.D.	0·059 ±·003	0·040 ±·002	0·037 ±·002	0·042 ±·002	0·064 ±·003	0·055 ±·002
	C.V.	13·9 ±·67 (9·8)†	9·2 ±·40	8·1 ±·35	9·3 ±·37	13·5 ±·54	11·6 ±·47

* During a part or the whole of these periods the cows were under summer grazing conditions.

Shorthorn cows in three weekly stages throughout a lactation period.

20 to 22	23 to 25	26 to 28	29 to 31	32 to 34	35 to 37	Mean of twelve 3 weekly periods, 2nd to 37th week	Mean of seven 3 weekly periods, 5th to 25th week
3·48 ±0·02	3·60 ±0·02	3·55 ±0·01	3·75 ±0·04	*3·96 ±0·06	*3·87 ±0·04	*3·53 ±0·01	3·32 ±0·01
0·11 ±0·01	0·13 ±0·01	0·10 ±0·01	0·23 ±0·02	0·43 ±0·04	0·25 ±0·03	0·28 ±0·03	0·24 ±0·01
3·2 ±0·3	3·6 ±0·4	2·9 ±0·3	6·2 ±0·6	10·8 ±1·1	6·4 ±0·7	7·83 ±0·24	7·32 ±0·29
4·30 ±0·02	4·33 ±0·03	4·39 ±0·03	4·65 ±0·04	4·68 ±0·08	4·60 ±0·04	4·59 ±0·02	4·42 ±0·02
0·16 ±0·02	0·18 ±0·02	0·21 ±0·02	0·29 ±0·03	0·53 ±0·05	0·29 ±0·03	0·38 ±0·01	0·38 ±0·02
3·7 ±0·4	4·0 ±0·4	4·9 ±0·5	6·2 ±0·6	11·4 ±1·2	6·4 ±0·7	8·18 ±0·25	8·36 ±0·33
2·84 ±0·04	3·02 ±0·02	*3·18 ±0·04	*2·92 ±0·09	*3·46 ±0·08	*4·16 ±0·10	*2·88 ±0·01	2·64 ±0·01
0·25 ±0·03	0·14 ±0·01	0·24 ±0·03	0·62 ±0·06	0·55 ±0·06	0·67 ±0·07	0·33 ±0·01	0·22 ±0·01
8·6 ±0·9	4·7 ±0·5	7·5 ±0·8	21·1 ±2·2	15·9 ±1·7	13·7 ±1·4	11·12 ±0·33	8·74 ±0·35
4·07 ±0·05	3·89 ±0·04	4·21 ±0·07	4·16 ±0·09	4·21 ±0·15	4·81 ±0·12	4·09 ±0·02	3·94 ±0·02
0·34 ±0·04	0·23 ±0·02	0·45 ±0·05	0·61 ±0·06	1·04 ±0·11	0·84 ±0·09	0·47 ±0·01	0·33 ±0·01
8·3 ±0·9	5·8 ±0·6	10·7 ±1·1	14·6 ±1·5	24·7 ±2·6	17·4 ±1·8	11·30 ±0·34	8·33 ±0·33
2·95 ±0·02	3·28 ±0·03	*3·11 ±0·06	*2·90 ±0·03	*3·15 ±0·05	*3·68 ±0·09	*2·96 ±0·01	2·83 ±0·01
0·11 ±0·01	0·21 ±0·02	0·44 ±0·05	0·21 ±0·02	0·33 ±0·03	0·61 ±0·06	0·34 ±0·01	0·25 ±0·01
3·6 ±0·4	6·3 ±0·7	14·1 ±1·5	7·0 ±0·7	10·5 ±1·1	16·6 ±1·7	11·09 ±0·33	8·36 ±0·33
3·42 ±0·05	3·54 ±0·04	4·31 ±0·04	4·20 ±0·04	4·36 ±0·07	5·45 ±0·15	4·17 ±0·02	3·73 ±0·02
0·32 ±0·03	0·24 ±0·03	0·30 ±0·03	0·28 ±0·03	0·45 ±0·05	1·02 ±0·11	0·43 ±0·01	0·32 ±0·01
9·3 ±1·0	6·9 ±0·7	7·0 ±0·7	6·7 ±0·7	10·3 ±1·1	18·7 ±1·9	9·86 ±0·30	8·47 ±0·33
3·51	3·61	3·79	3·76	3·97	4·43	3·70	3·48
0·22	0·19	0·29	0·37	0·56	0·61	0·37	0·29
6·1	5·2	7·9	10·3	13·9	13·2	9·9	8·2

fat yield of an average cow during each month of the year.

July	Aug.	Sept.	Oct.	Nov.	Dec.	Mean value for the 12 months
13·8 ±·06	12·4 ±·05	14·5 ±·06	12·8 ±·05	14·3 ±·05	13·6 ±·04	14·1 ±·01
0·78 ±·04	0·73 ±·04	0·88 ±·05	0·64 ±·03	0·73 ±·04	0·56 ±·02	0·73 ±·01
5·9 ±·29	6·0 ±·30	6·1 ±·31	5·0 ±·26	5·1 ±·27	4·4 ±·19 (4·2)†	5·4 ±·07
9·5 ±·07	8·1 ±·06	10·0 ±·07	8·0 ±·05	8·7 ±·03	8·2 ±·04	9·1 ±·01
0·88 ±·04	0·68 ±·03	0·85 ±·04	0·66 ±·03	0·39 ±·02	0·70 ±·03	0·68 ±·01
9·7 ±·48	8·4 ±·42	9·1 ±·46	7·8 ±·40	4·6 ±·24	8·5 ±·36 (6·9)†	7·6 ±·07
11·6 ±·08	10·2 ±·07	12·2 ±·09	10·4 ±·05	11·5 ±·04	10·9 ±·04	11·6 ±·01
0·83 ±·04	0·71 ±·04	0·87 ±·04	0·65 ±·03	0·56 ±·03	0·63 ±·03	0·71 ±·01
7·8 ±·39	7·2 ±·36	7·6 ±·39	6·4 ±·33	4·8 ±·25	6·4 ±·27 (5·6)†	6·5 ±·08
0·46 ±·004	0·43 ±·004	0·49 ±·004	0·44 ±·004	0·45 ±·003	0·47 ±·003	0·48 ±·001
0·063 ±·003	0·058 ±·003	0·059 ±·003	0·060 ±·003	0·046 ±·002	0·055 ±·002	0·053 ±·001
13·5 ±·67	13·7 ±·68	12·5 ±·65	12·8 ±·66	10·0 ±·52	12·0 ±·50 (8·8)†	11·2 ±·14
0·42 ±·005	0·35 ±·004	0·43 ±·006	0·37 ±·004	0·38 ±·003	0·37 ±·003	0·40 ±·001
0·078 ±·004	0·057 ±·003	0·077 ±·004	0·050 ±·003	0·042 ±·002	0·045 ±·002	0·054 ±·001
18·7 ±·93	15·9 ±·79	18·9 ±·97	13·8 ±·71	11·2 ±·59	13·2 ±·55 (10·9)†	13·6 ±·18
0·44 ±·005	0·39 ±·004	0·46 ±·005	0·40 ±·004	0·41 ±·003	0·42 ±·003	0·44 ±·001
0·070 ±·003	0·058 ±·003	0·068 ±·004	0·055 ±·003	0·044 ±·002	0·050 ±·002	0·054 ±·001
16·1 ±·80	14·8 ±·73	15·7 ±·81	13·3 ±·69	10·6 ±·55	12·6 ±·53 (9·9)†	12·4 ±·16

† Figures obtained after elimination of cows milked by machine.

inversely to the milk yield. On comparing the C.V. of the morning and those of the evening milking, it will be seen that the former tend to be distinctly lower, this being a further indication of the tendency for high milk yields to lower the C.V. The interpretation of this fact is of value in pointing to the causes of day to day variation in milk yields. If these variations were due to factors affecting the actual rate of milk secretion or to changes in the intervals between milkings, the variability should be proportional to the yield, thereby producing a constant coefficient of variability. If, however, the variations were due to varying quantities of milk retained in the udders of cows at milking time, then the S.D. might be expected to be unaffected by milk yield and the C.V. to vary inversely to it. The fact that this second alternative obtains suggests incomplete milking as a possible cause of day to day variability. It is not suggested that incomplete milking is necessarily the fault of the milker, probably it is more often due to retention of milk by cows due to nervous reaction resulting from discomfort, ill health or other causes.

The high variability of milk yield during the first few weeks after calving is not unexpected, since cows may not be in perfect health during this period and are usually nervous owing to the loss of their calves. Other figures not published here indicate that it is during the first ten days after calving that the greatest variations take place.

Attention may now be drawn to variations in the yield of fat shown in Table II section (*b*). As in the case of milk yields it will be observed that the S.D. are high during the first month of lactation, but there is a tendency towards a gradual fall in the S.D. with advancing lactation. The result is that there are only slight indications of a rise in the C.V. with the advance of lactation, certainly not of a very significant rise.

Applying the same interpretation as is offered above in respect of milk yields, it can be argued that it would appear that variations in fat yield may be due to varying rates of secretion of fat as well as to incomplete milking. It is not desired to emphasise this point too much, however, because of the lack of significance in the figures on which the argument is based.

Comparison between the C.V. of milk yield and fat yield shows that fat varies approximately twice as much as milk; the reason for this may be found largely in the fact that the last milk drawn from a cow is considerably richer in fat than the total milk drawn at a complete milking, the result being that if a proportion of milk is retained in the udder at any milking the proportion of fat retained is correspondingly greater. Again, if, as has been suggested, there is a greater tendency

Table II. *Showing the comparative variability of milk and fat yield for an average cow during each month of a lactation.*

		Month of lactation 1	2	3	4	5	6	7	8	Mean value for the 8 months
(a) **Milk yield**										
a.m. milkings	Mean (lb.)	18·5 ±·05	18·6 ±·04	17·4 ±·04	15·1 ±·04	13·6 ±·03	12·8 ±·04	11·4 ±·04	10·3 ±·03	14·7 ±·01
	S.D.	1·09 ±·05	0·76 ±·03	0·81 ±·03	0·82 ±·03	0·69 ±·02	0·73 ±·03	0·79 ±·03	0·51 ±·02	0·77 ±·01
	C.V.	5·9 ±·20	4·1 ±·14	4·5 ±·16	5·6 ±·19	5·0 ±·17	5·7 ±·20	6·9 ±·24	5·1 ±·17	5·34 ±·07
p.m. milkings	Mean (lb.)	13·0 ±·06	12·6 ±·04	11·3 ±·04	9·7 ±·03	8·8 ±·04	7·8 ±·03	7·3 ±·04	6·4 ±·02	9·6 ±·01
	S.D.	1·26 ±·04	0·80 ±·03	0·83 ±·03	0·60 ±·02	0·80 ±·03	0·59 ±·02	0·74 ±·03	0·49 ±·02	0·76 ±·01
	C.V.	9·7 ±·33	6·4 ±·22	7·0 ±·24	6·0 ±·21	8·9 ±·31	7·6 ±·26	9·8 ±·34	8·0 ±·28	7·88 ±·10
Mean values for the a.m. and p.m. milkings	Mean (lb.)	15·7 ±·07	15·6 ±·04	14·4 ±·04	12·4 ±·03	11·2 ±·04	10·3 ±·03	9·3 ±·04	8·3 ±·02	12·2 ±·01
	S.D.	1·17 ±·04	0·78 ±·03	0·82 ±·03	0·71 ±·02	0·75 ±·03	0·66 ±·02	0·76 ±·03	0·50 ±·02	0·77 ±·01
	C.V.	7·8 ±·27	5·2 ±·18	5·8 ±·20	5·9 ±·20	7·0 ±·24	6·7 ±·23	8·3 ±·28	6·6 ±·23	6·61 ±·08
(b) **Yield of fat**										
a.m. milkings	Mean (lb.)	0·614 ±·003	0·575 ±·003	0·563 ±·003	0·502 ±·003	0·469 ±·003	0·445 ±·002	0·404 ±·002	0·381 ±·002	0·494 ±·001
	S.D.	0·068 ±·002	0·056 ±·002	0·070 ±·002	0·054 ±·002	0·055 ±·002	0·042 ±·001	0·049 ±·002	0·045 ±·002	0·055 ±·001
	C.V.	11·2 ±·39	9·9 ±·34	12·6 ±·43	11·0 ±·38	11·7 ±·40	9·5 ±·32	12·0 ±·41	11·8 ±·41	11·2 ±·14
p.m. milkings	Mean (lb.)	0·591 ±·005	0·548 ±·003	0·495 ±·003	0·418 ±·002	0·388 ±·003	0·349 ±·002	0·329 ±·003	0·297 ±·002	0·427 ±·001
	S.D.	0·099 ±·003	0·070 ±·002	0·064 ±·002	0·048 ±·002	0·057 ±·002	0·048 ±·002	0·053 ±·002	0·042 ±·001	0·060 ±·001
	C.V.	17·2 ±·59	12·6 ±·43	12·6 ±·43	11·2 ±·39	14·8 ±·51	13·8 ±·48	16·1 ±·56	14·7 ±·51	14·1 ±·17
Mean values for the a.m. and p.m. milkings	Mean (lb.)	0·603 ±·004	0·562 ±·003	0·529 ±·003	0·460 ±·003	0·429 ±·003	0·397 ±·002	0·367 ±·003	0·389 ±·002	0·467 ±·001
	S.D.	0·084 ±·003	0·063 ±·002	0·067 ±·002	0·051 ±·002	0·056 ±·002	0·045 ±·002	0·051 ±·002	0·043 ±·001	0·058 ±·001
	C.V.	14·2 ±·49	11·3 ±·39	12·6 ±·43	11·1 ±·38	13·3 ±·46	11·7 ±·40	14·1 ±·49	13·2 ±·45	12·7 ±·16
(c) **Fat percentage**										
a.m. milkings	Mean (% fat)	3·3 ±·02	3·1 ±·01	3·3 ±·01	3·4 ±·01	3·5 ±·02	3·6 ±·01	3·6 ±·02	3·8 ±·02	3·45 ±·005
	S.D.	0·36 ±·01	0·28 ±·01	0·30 ±·01	0·24 ±·01	0·34 ±·01	0·28 ±·01	0·36 ±·01	0·40 ±·01	0·32 ±·004
	C.V.	12·3 ±·42	8·8 ±·30	9·2 ±·32	7·4 ±·25	8·0 ±·28	7·5 ±·26	9·8 ±·34	10·1 ±·35	9·13 ±·11
p.m. milkings	Mean (% fat)	4·6 ±·03	4·4 ±·02	4·5 ±·02	4·4 ±·02	4·4 ±·02	4·5 ±·01	4·6 ±·02	4·7 ±·02	4·5 ±·007
	S.D.	0·63 ±·02	0·38 ±·01	0·40 ±·01	0·33 ±·01	0·39 ±·01	0·29 ±·01	0·42 ±·01	0·44 ±·02	0·41 ±·005
	C.V.	15·0 ±·52	9·2 ±·32	9·1 ±·31	7·7 ±·27	9·0 ±·31	6·7 ±·23	8·3 ±·29	8·7 ±·30	9·22 ±·11
Mean values for the a.m. and p.m. milkings	Mean (% fat)	4·0 ±·02	3·8 ±·02	3·9 ±·02	3·9 ±·01	4·0 ±·02	4·0 ±·01	4·1 ±·02	4·3 ±·02	4·0 ±·006
	S.D.	0·49 ±·02	0·33 ±·01	0·35 ±·01	0·29 ±·01	0·36 ±·01	0·29 ±·01	0·39 ±·01	0·42 ±·01	0·37 ±·005
	C.V.	14·2 ±·49	9·0 ±·31	9·2 ±·32	7·6 ±·26	8·5 ±·29	7·1 ±·24	9·1 ±·31	9·4 ±·32	9·18 ±·11

for the rate of secretion of fat to vary more from day to day than the rate of secretion of milk, this provides an additional reason for a greater variability of fat.

Table II section (*c*) deals with fat percentages, and this section is included chiefly to give results comparable with Table I, the essential difference being that in Table II (*c*) any seasonal effect is spread over the whole period and the slightly higher mean C.V. in Table II (*c*) are probably due to the seasonal effects. It will be observed that the C.V. at the morning milkings closely approximate to those of the evening milkings during each month of lactation. Conclusions are difficult to draw from these figures regarding the secretion of fat or of milk, since as has been pointed out by Gaines (2) "Fat percentage is merely a mathematical expression of the ratio (× 100) of the average rate of fat secretion to the average rate of milk secretion."

Day to day variability as affected by season of the year.

The data used for the preparation of Table II were next classified to show the variability of milk and fat yields as affected by season of the year, the first month of lactation being omitted in every case owing to its abnormality. The results are shown in Table III, section (*a*) dealing with milk yield, and section (*b*) with yield of fat during each month of the year. It will be observed that the greatest variability occurs during the month of May, and all the summer months May to October tend to yield higher figures than the winter months. December and January produced much higher figures than any of the other winter months and, when seeking to discover whether the cause was due to abnormalities in the data, it was found that the cows showing the greatest variability for these two months were animals which had been milked by a newly installed milking machine. The machine was first used in December and for two months or so some of the cows and the machine operator had not become thoroughly acquainted with the change of milking methods. It was decided to eliminate all machine-milked cows and the amended figures are shown in brackets under the original figures in Table II. It will be observed that the final results were much more uniform.

The averages of the standard deviations for the six winter and six summer months respectively were:

	Standard deviations of	
	Milk yield	Fat yield
6 summer months	0·82	0·062
6 winter months	0·59	0·045

The greater variability during the summer months, especially May, was probably due to the lack of control as to diet and exercise when cows were grazing as compared with winter conditions, and this may be a reason why the depression in the fat percentage of summer milk is often over-estimated. When fat tests of milk are made, there is a tendency for the farmer to hear nothing of the samples which are well above the 3 per cent. standard, but samples below the standard always receive attention, *e.g.* if the average morning milk from a herd tests 3·3 per cent. of fat and the cows owing to summer conditions show considerable variability from day to day, a proportion of the morning milkings will yield a mixed milk below 3 per cent., whereas if the variability is low as in the winter time, few if any morning samples would test lower than the 3 per cent. standard. This point should be kept in mind when farmers are accused of adulterating their milk and in the event of an "Appeal to the Cow" the results may be very misleading if a single milking is relied upon.

APPLICATION OF RESULTS.

It is most important to realise that the figures given in Tables I, II and III apply only when cows are managed and milked in a regular manner, the extent to which the quantity and quality of milk can be influenced by bad management or inefficient milking is practically unlimited; where such conditions exist the figures given here are not applicable. It might be thought possible that figures calculated from a single herd can have no wider application than that herd, but comparison with results from a few other farms indicate that the figures presented are fairly representative of many well-managed herds.

By employing certain formulae the variabilities shown in Tables I, II and III may be utilised for calculating the extent and chances of error which are likely to occur when the fat content of milk is obtained from:

(*a*) A sample from a single cow at a single milking and it is assumed that such a sample represents a fair average of other corresponding milkings a few days before or after.

(*b*) A mixed sample composed of the milk of any given number of cows at one milking and it is assumed to represent fairly similar milkings a few days before or after.

(*c*) A succession of samples taken at regular intervals throughout the lactation of a single cow and the average of these samples is assumed to state accurately the average fat percentage which would be obtained as a result of daily testing.

The two simple and well-known formulae which it is suggested may be used are as follows:

(1) The probable value of a single variate

$$= \pm \text{Standard deviation} \times 0{\cdot}67.$$

(2) The probable value of the mean of a number of variates

$$= \pm\, 0{\cdot}67 \, \frac{\text{Standard deviation}}{\sqrt{\text{Number of variates}}}.$$

The application of these formulae may be illustrated by an example:

Take a hypothetical cow which had calved about 3 months and at the evening milking on November 10th yielded 10 lb. milk containing 5 per cent. fat, *i.e.* a yield of 0·5 lb. fat. It is often asked what yield of fat might be expected from this cow at any other evening milking (similar milking intervals) between say November 1st and 20th.

Table III (*b*) shows a standard deviation for evening milkings in November of 0·042, and the probable error becomes

$$0{\cdot}042 \times 0{\cdot}67 = 0{\cdot}03.$$

So that the yield of fat for the cow on any day between November 1st and 20th may be expressed as $0{\cdot}5 \pm 0{\cdot}03$, which would mean that on about 10 occasions of the evening milkings the yield of fat would lie between 0·47 and 0·53 lb. and on about 10 occasions it would lie outside this range.

Again if $50 \times 0{\cdot}5$, *i.e.* 25 lb. fat, represented the yield of fat by a herd of 50 cows on the evening of November 10th, it might be asked what yield may be expected from the same herd at any other evening between November 1st and 20th if milked at the same intervals and efficiently managed.

Average yield of herd 0·5 lb. fat. Standard deviation 0·042.

Using the second formula

$$\cdot 67 \, \frac{\cdot 042}{\sqrt{50}} = \cdot 67 \, \frac{\cdot 042}{7{\cdot}05} = \cdot 004.$$

The average yield per cow $= 0{\cdot}5 \pm 0{\cdot}004$.

Therefore the total yield of 50 cows $= 25$ lb. $\pm\, 0{\cdot}2$.

This suggests the probability that at about 10 of 20 evening milkings the yield of fat of this herd of 50 cows may be expected to be between 24·8 and 25·2 lb. and at the remaining evenings the yield will be outside this range. A consistent result such as is given here cannot be expected if a rapid falling off in milk yield has taken place during the period, but such a condition is unusual in a well-managed herd.

The same formulae may be used to calculate the probable error when butterfat tests of a cow have been taken at six weekly and at shorter

intervals throughout a lactation period in order to decide the animal's production record. In the example below fat percentage is dealt with instead of weight of fat and the calculations are based on the coefficient of variability in order to show the application of this figure.

Assuming the exact fat percentage yielded by a cow based on daily tests to be 4 per cent., and taking a normal coefficient of variability throughout a lactation period as 10 (see Table II (*c*)), the standard deviation becomes $\frac{10 \times 4{\cdot}0}{100} = 0{\cdot}40$. If the cow were tested for fat percentage on 8 days during the lactation (16 milkings), the probable error of the estimated fat percentage for the lactation period becomes

$$\pm \cdot 67 \frac{\cdot 4}{\sqrt{16}} = \pm \cdot 067,$$

i.e. the chances are even that 8 complete day tests will give a result between 3·93 and 4·07 per cent.

If tested on 32 days (at equal intervals) during the lactation (64 milkings), the probable error of the result becomes

$$\pm \cdot 67 \frac{\cdot 4}{\sqrt{64}} = \pm \cdot 034,$$

i.e. the chances are even that 32 complete day tests will give a result between 3·966 and 4·034.

Assuming the figures to follow the normal law of errors the range within which 99 per cent. of the results will fall is nearly four times as wide as the 50 per cent. range, so that in the examples mentioned above 99 per cent. of the calculated results from a 4 per cent. lactation record will:

(*a*) In the case of cows tested on 8 days fall between 3·7 and 4·3 per cent.

(*b*) In the case of cows tested on 32 days fall between 3·87 and 4·13 per cent.

When this theoretical method of calculating the errors was tested against actual fat test results calculated from cows tested daily throughout a lactation period, it was found that the errors in practice corresponded closely with the expected errors.

On comparison with the figures presented by Sheeny (3), it appears that the cows in the present investigation varied somewhat less and are therefore liable to less error by infrequent sampling. It is not suggested that the sampling of cows on 8 days only during a lactation period will always yield such accurate results as those above. There are other factors apart from the normal variability in yield of fat which can cause

errors, *e.g.* the yield of a cow can be seriously affected for a single day by the deliberate design of the owner or cowman by such means as changing the hours of milking, also a cow tested for the first time shortly after calving is subject to a greater error than a cow tested three weeks after calving. Most of the errors due to these causes, however, can be eliminated by the application of suitable regulations governing the system of testing, whereas the normal variations in yield from day to day cannot be eliminated and can only be reduced by taking the average of a number of tests taken at regular intervals during the lactation period.

Day to day variability of 24 hour yields.

It was considered possible that when a fall in milk yield occurred at one milking the lost milk might be recovered at the following milking and if this occurred to any appreciable extent the method of calculating the error based on morning milkings only or evening milkings only would be unsound.

Comparison was, therefore, made between variability of the fat percentage of the three cows for which daily records were available.

(*a*) When the fat percentage for 24 hours was calculated from a morning yield plus the subsequent evening yield.

(*b*) When the fat percentage was calculated from an evening yield plus the subsequent morning yield.

The mean of the standard deviations calculated for morning and evening samples when treated separately was 0·344. The reduction in this figure which would result from chance improvement due to averaging two variates diminishes it to $\frac{\cdot 344}{\sqrt{2}} = \cdot 243$.

The observed standard deviation calculated for a 24 hour yield consisting of a morning yield plus the subsequent evening yield was $0{\cdot}218 \pm 0{\cdot}007$.

The observed standard deviation calculated for a 24 hour yield consisting of an evening yield plus the subsequent morning yield was $0{\cdot}243 \pm 0{\cdot}008$.

It will be seen that the observed standard deviation for a 24 hour percentage made up of an evening plus the subsequent morning yield is identical with the calculated figure, *i.e.* 0·243, and that the standard deviation of a 24 hour percentage made up of a morning plus the subsequent evening yield is slightly lower, *i.e.* 0·218. These figures are interesting from two points of view, firstly that the excellent agreement between the figures suggests that the method adopted of a single milking as a unit in the calculations for this study is quite sound, while the second

point of view is connected with the system of milk recording in this country. When taking milk records it is the practice for a 24 hour yield to be made up of milkings taking place between noon one day to noon the next day, and where twice daily milking is practised this means an evening and the subsequent morning milkings. It would appear from the above figures that in herds where the milking intervals are unequal a slightly less variable record of fat percentage would be obtained if the 24 hour records commenced at the morning milkings; the difference in the two figures is so small however, that no great advantage is likely to be gained by a change of system.

SUMMARY.

1. The day to day variability in the yield of milk and fat of cows is affected by many factors, two of which are stage of lactation and season of the year. Variability is high during the first month of lactation (particularly during the first few days after the calf is weaned), but after this a fairly constant variability may be expected. Under the conditions of management in the herd under discussion and probably in most herds in Southern England variability is highest during the month of May and also tends to be higher during the summer than the winter months.

2. A method is presented of calculating the variability in the yield of mixed milk from a herd of cows and the error to be expected when milk records and fat percentages of individual cows are calculated from a few samples.

3. When cows are milked twice daily at unequal intervals the yield of milk obtained in 24 hours is slightly less variable if a morning milk yield is added to the subsequent evening yield, than if an evening yield is added to the subsequent morning yield.

I am deeply indebted to Mr E. Capstick for considerable help during the early stages of this work, also to Sir Gilbert Walker and Mr J. Clatworthy for most helpful suggestions in connection with the statistical work involved.

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THE EFFECT OF HEAT ON MILK.

(A) ON THE COAGULABILITY BY RENNET.

(B) ON THE NITROGEN, PHOSPHORUS AND CALCIUM CONTENT.

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(With Two Text-figures.)

A great deal has been written during the last few years upon the effects—deleterious or otherwise—of heat upon milk. That certain changes are brought about in the composition of milk by heating seems to be generally agreed, but enquiries into the nature of these changes have generally been made only at one or two particular temperatures. It was felt that possibly results of more value might be obtained by studying the effect of heating milk over a wide range of temperature, and it was decided to investigate the effect of heat upon (*a*) the time of coagulation by rennet, and (*b*) the solubility of the calcium, phosphorus and nitrogen present.

One of the most fundamental properties of milk is its coagulability by rennet and in the manufacture of cheese the production of a satisfactory curd from the milk on the addition of rennet is one of the most important factors. Upon the nature of the coagulum formed in the early stages of the manufacture of any cheese depends to a great extent the quality of the final product. For the production of a good cheese, it is necessary that a firm coagulum shall be produced by the addition of a definite quantity of rennet to a known volume of milk at a standard temperature. If for any reason, coagulation is delayed, and the resulting coagulum is soft, whey will not drain freely from the curd, and the resulting product will generally be poor both in texture and flavour.

In practice it has been found by cheese makers that a soft unsatisfactory curd which is difficult to drain is always produced when the usual quantity of rennet is added to milk which has been pasteurised, but it has also been found that the addition of lime water or a solution of calcium chloride to the milk before the addition of rennet results in

the formation of a firm curd from which the whey drains in an almost normal manner.

These facts which have been known to cheese makers for many years seem to indicate three things:

(1) That the composition of the milk has been altered in some definite way by pasteurisation.

(2) That the constituents which have been affected are amongst those which are concerned with the formation of a coagulum on the addition of rennet.

(3) Since the production of an almost normal curd results on the addition of a solution of a calcium salt to the milk, it is reasonable to suppose that the heating has affected particularly the calcium salts of the milk.

METHOD OF EXPERIMENT—RENNET TESTS.

The extent to which heating milk affects the time of coagulation by rennet was determined in the following way. Several pints of Grade A morning milk were taken into a sterile can and well mixed. After determining the acidity by titration with ninth-normal caustic soda using phenolphthalein as indicator, a quantity was taken out and transferred to a second sterile can. The milk remaining in the original can was then heated in a water bath to the required temperature, the time necessary being never less than 8 minutes nor greater than 20 minutes, at which it was maintained for half an hour. During the whole time, the milk was stirred mechanically, the vessel being closed with a lid through which a rod carrying stirring vanes was passed. Two thermometers were also passed through the lid of the pasteurising vessel so that the temperatures of the milk, both at the bottom and top of the vessel, could be observed.

The milk having been kept for half an hour at the required temperature, it was cooled rapidly by passing over a previously sterilized cooler into a sterile can, and samples were withdrawn periodically to determine the time of coagulation by rennet. Samples were also taken from the raw milk for comparison.

The rennet test was made in a special apparatus which was designed originally by the late Mr John Benson, and consists of an insulated cylindrical metal vessel mounted on three legs, with an agate nozzle with an opening of one millimetre fixed into the base. There is also a well fitting and insulated lid.

The rennet solution was prepared by diluting a volume of commercial

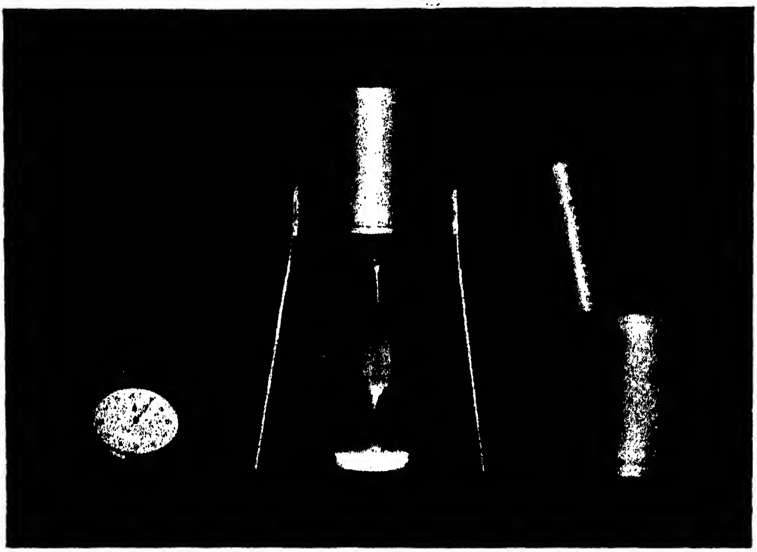

Fig. 1.

Fig. 2.

rennet with twice its volume of tap water. Two hundred and fifty cubic centimetres of the milk were measured and brought to a temperature of 84° F., transferred quickly to the previously warmed insulated vessel (the opening of the nozzle being closed with a pin), three cubic centimetres of the diluted rennet solution added and the mixture stirred rapidly for ten seconds. At the end of this time the lid was put on and the pin removed. At first the milk runs rapidly in a fine stream through the agate nozzle; later the stream becomes intermittent and finally ceases at the moment when coagulation is complete.

This method, as at first designed, had one drawback, namely, that it was extremely difficult to determine the time of falling of the last drop. This difficulty has now been overcome by the use of a piece of blackened glass, which is placed underneath the nozzle in a slanting position so that the stream of milk must run over it, and by carefully watching, it is possible to determine the moment when coagulation begins by the appearance on the dark surface of small particles of solid matter.

The time of the rennet test, determined by a stop watch, is taken from the moment of the addition of the rennet to the milk to the moment when the particles of casein make their appearance on the glass. The experimental error of the method is not more than ± 4–5 seconds.

Figs. 1 and 2 are photographs showing the apparatus as above described at the beginning and the end of a test. The solid particles of casein appearing on the blackened glass show well in Fig. 2.

The results are tabulated in Table I.

During the course of some preliminary experiments it was found that the time of coagulation with rennet of milk which had been heated did not remain constant but became gradually longer over a period of some hours after the heating was concluded, but that in all cases the conditions which resulted in this slowing down of the rennet test appeared to become stabilised at the end of five hours. The experiments were actually carried out over a period of 30 hours, but since no definite changes take place during the last 25, the results obtained during the first five hours only are shown.

From a consideration of Table I it is seen that this "slowing down" effect already mentioned is well marked even with milk which has been heated only to a temperature of 106–110° F., the time of coagulation with rennet changing from 2 minutes 50 seconds to 3 minutes 17 seconds during five hours, this difference of 27 seconds being well outside the limits of experimental error; the differences with milk heated to 115–

Table I.

Temperature of heating. ° F.	Acidity of raw milk ($N/9$ NaOH): Original. % lactic acid	Acidity of raw milk ($N/9$ NaOH): After 5 hours. % lactic acid	Acidity of heated milk ($N/9$ NaOH): Immediately after heating. % lactic acid	Acidity of heated milk ($N/9$ NaOH): 5 hours after heating. % lactic acid	Rennet tests of raw milk: First test min. sec.	Rennet tests of raw milk: Test after 5 hours min. sec.	Rennet tests of heated milk: First test immediately after cooling min. sec.	Second test 10 mins. after first test min. sec.	Third test ¾ hour after first test min. sec.	Fourth test 1½ hours after first test min. sec.	Fifth test 2½ hours after first test min. sec.	Sixth test 3½ hours after first test min. sec.	Seventh test 5 hours after first test min. sec.
105–110	0·163	0·161	0·160	0·160	3 14	3 16	2 50	2 55	3 4	3 13	3 16	3 17	3 17
115–120	0·163	0·164	0·165	0·164	2 51	2 50	2 46	2 50	2 55	2 59	3 14	3 14	3 12
125–129	0·171	0·170	0·169	0·170	3 0	3 1	2 45	2 56	3 5	3 15	3 5	3 9	3 8
135–141	0·178	0·176	0·168	0·168	2 55	3 0	2 35	2 46	2 57	3 5	3 30	3 32	3 27
145–151	0·150	0·150	0·146	0·145	4 0	3 57	4 4	4 15	4 46	5 6	5 7	5 4	5 5
155–160	0·162	0·160	0·156	0·155	3 24	3 24	4 45	5 7	5 30	5 40	6 5	6 6	6 3
165–171	0·178	0·176	0·174	0·176	2 30	2 34	7 35	9 10	9 40	11 40	14 45	14 30	14 50
175–178	0·152	0·156	0·150	0·154	2 42	2 45	8 0	9 15	—	11 0	13 0	12 45	12 49
185–189	0·169	0·166	0·160	0·164	2 55	2 57	7 40	9 15	—	11 55	13 55	13 45	14 10
195–198	0·161	0·160	0·155	0·151	3 25	3 23	11 25	14 35	No coag.				
205–209	0·162	0·163	0·160	0·160	3 10	3 12	No coag.						

120° F. and 125–129° F. are of the same order, viz. 26 and 23 seconds. From 135–141° F. to 195–198° F. however the differences become much greater, and at 195–198° F. a thickening only was produced in the milk by the addition of rennet half an hour after heating, while at 205–209° F. no coagulation was obtainable at any time after heating and subsequent cooling.

It will be noticed that the change in the rennet test of the raw milk during five hours is never outside experimental error, only once being as great as 5 seconds.

One other point is brought out by a consideration of Table I. To whatever cause the slowing down of the rennet test of the heated milk is due, it is obviously not a change in titratable acidity, the change in the titratable acidity of the heated milk being never more than 0·004 per cent. which is well within the limits of experimental error; neither is it due to an initial change in the acidity due to the heating, since the difference between the acidity of the raw milk at the beginning of the experiment and that of the heated immediately after cooling at the time of the first rennet test is only once as high as 0·01 per cent.

In addition to the "slowing down" of the rennet test in heated milk, two other interesting points have arisen during the present experiments and are evident from the Table. The first of these is the fact that milk which has been heated to temperatures varying from 105–140° F. for half an hour, and then quickly cooled, has a slightly quicker rennet test when this test is made immediately after cooling than the test made on raw milk, although the test after five hours is always slower; whereas with milk which has been heated to 145° F. and above, the first test and all subsequent ones are slower than with the raw milk.

The second point is illustrated in the rennet tests of milk heated to 165–171° F., 175–178° F. and 185–189° F., which all show a slight but definite quickening at about four hours after heating, while all subsequent tests are slower. This particular phenomenon has been repeatedly noticed by the authors, not only with milk heated to these higher temperatures but often at much lower ones, although it did not occur at the lower temperatures in the particular series shown here.

It is proposed to investigate the causes of these phenomena in future experiments.

The facts which have been demonstrated in the experiments outlined above, show quite definitely that the constituents of milk which has been heated for half an hour at any temperature between 105° F. and 209° F. have been changed in such a way that the reaction with rennet

is no longer normal, but becomes increasingly abnormal with increased temperatures of heating.

Moreover it has also been shown that the change is progressive for five hours after heating, which is an important factor in cheese making, as the nature of the coagulum produced by renneting and its condition during the hours immediately following are the decisive factors in the quality of a cheese.

Effect of heat upon the nitrogen, phosphorus and calcium content.

Earlier in this paper it was stated that a more or less normal coagulum could be produced by renneting heated milk if a solution of a calcium salt were added to the milk previous to the addition of the rennet. This fact, which is well known, seems to indicate that in the process of heating, the calcium salts of the milk have either been removed from the milk or else altered in such a way that they cease to be effective in the process of coagulation.

Many workers have investigated this subject, amongst whom may be mentioned Palmer (1) who says, "The fact that pasteurisation of milk retards the coagulability of the casein by rennet and the fact that this property can be restored by the addition of calcium chloride to the milk, has been presented in support of the view that heat changes some of the soluble calcium salts to an insoluble form. The experimental evidence for such a change is, however, contradictory." In support of this statement he mentions the work of Soldner (2), Boekhout and De Vries (3), Purvis, Brehant and McHattie (4) and Grosser (5), all of whom have come to the conclusion that when milk is boiled, a precipitate of a portion of the calcium phosphate occurs. Rupp (6), however, found no change in the calcium content of milk which had been held for thirty minutes at a temperature of 68·3° C. (154·9° F.) and further states that the phosphoric acid content of the serum of both raw milk and that which had been pasteurised at 155° F. for thirty minutes was the same. Milroy (7), after holding fresh milk just below boiling point for one hour, filtered through an ordinary filter and noted a decline in the calcium, while Magee and Harvey (8) found that about 26 per cent. of the total calcium was found in diffusible form in fresh milk, about 20 per cent. in milk pasteurised at 65° C. for thirty minutes and about 15 per cent. in milk kept at boiling point for an hour. These writers do not, however, investigate changes at any other temperatures.

Bell (9) using fresh skim-milk and filtering through a Pasteur Chamberlain filter comes to the conclusion that there is a loss in the soluble calcium and phosphorus contents which are definitely measurable in milk which has been heated to 170° F. or higher for thirty minutes, while Diffloth (10) found a decrease in the soluble phosphates amounting to 25·9 per cent. on heating milk to 140° F. for thirty minutes.

It is obvious that the total amounts of calcium, phosphorus and nitrogen in milk cannot be altered by the application of heat, although the differences observed in heated milk render it probable that some change in the solubility of one or more of their salts may be effected. Since the opinions on this point appeared as cited in the preceding paragraph not to be unanimous it was decided to determine the total and diffusible calcium, phosphorus and nitrogen content of raw milk and milk which had been heated for half an hour over the range of temperatures already mentioned. The following technique was adopted. Estimations of the total calcium and phosphorus contents were made in duplicate on the ash of 25 c.c. of milk which were measured by a standard pipette and then weighed. The calcium was estimated by precipitation as oxalate, solution in H_2SO_4 and titration of the free oxalic acid by tenth-normal potassium permanganate solution. The phosphate was determined on the same quantity of milk as magnesium pyrophosphate and the total nitrogen on approximately five grams by the Kjeldahl method.

DIFFUSIBLE CALCIUM, PHOSPHORUS AND NITROGEN.

A number of parchment diffusion capsules were standardised by diffusing through them the same quantities of milk into a 3 per cent. solution of sodium chloride, and estimating the calcium in the saline solution.

After having experimented with a large number, eight were selected, and divided into two sets of four, the results for each set agreeing very closely. These eight capsules were used throughout the experiments and were standardised on several occasions.

After the milk had been heated as already described, it was kept in sterile cans for five hours, during which time samples were taken for the rennet tests; at the end of five hours four quantities each of 25 c.c. were taken from the raw milk and four similar ones from the heated milk and pipetted into the eight capsules. As the same standard pipette was used throughout the weight of milk in each capsule was known. Each capsule was plugged lightly with cotton wool and

Table II.

Temperature of heating ° F.	Calcium content: Total calcium in raw milk %	Total calcium in heated milk %	Diffusible calcium in raw milk %	Diffusible calcium in heated milk %	Phosphorus content: Total phosphorus in raw milk %	Total phosphorus in heated milk %	Diffusible phosphorus in raw milk %	Diffusible phosphorus in heated milk %	Nitrogen content: Total nitrogen in raw milk %	Total nitrogen in heated milk %	Diffusible nitrogen in raw milk %	Diffusible nitrogen in heated milk %
105–110	0·116	0·114	0·0319	0·0321	0·099	0·103	0·038	0·040	0·500	0·491	0·024	0·024
115–120	0·126	0·126	0·0326	0·0330	0·109	0·110	0·038	0·034	0·514	0·522	0·022	0·018
125–129	0·127	0·125	0·0343	0·0326	0·114	0·111	0·036	0·037	0·560	0·569	0·016	0·017
135–141	0·123	0·125	0·0320	0·0316	0·111	0·113	0·039	0·038	0·515	0·575	0·018	0·019
145–151	0·122	0·122	0·0295	0·0267	0·104	0·102	0·030	0·030	0·567	0·575	0·022	0·022
155–160	0·119	0·118	0·0334	0·0293	0·103	0·103	0·033	0·034	0·508	0·503	0·020	0·022
165–171	0·126	0·127	0·0332	0·0303	0·113	0·112	0·037	0·038	0·481	0·491	0·024	0·023
175–178	0·125	0·126	0·0383	0·0339	0·105	0·105	0·037	0·033	0·505	0·507	0·029	0·028
185–189	0·134	0·133	0·0328	0·0282	0·109	0·111	0·034	0·032	0·571	0·575	0·020	0·020
195–198	0·130	0·134	0·0329	0·0292	0·108	0·109	0·031	0·028	0·570	0·577	0·027	0·027
205–209	0·126	0·128	0·0376	0·0343	0·110	0·109	0·037	0·036	0·513	0·527	0·019	0·019

floated in 150 c.c. of the 3 per cent. saline solution. Diffusion was allowed to go on for 18 hours. At the end of that time, each capsule was removed, drained for 30 seconds, and the calcium, phosphorus and nitrogen which had diffused into the saline were estimated by the method already outlined.

The diffusate from each set of four capsules was made up to 1000 c.c. and six volumes of 150 c.c. were removed, so that determinations of the diffusible calcium, phosphorus and nitrogen were made in duplicate on quantities of diffusate corresponding to about 23 grams of milk. (On two occasions only—milk heated to 195–200° F. and 185–190° F.—was the quantity less than this, and in these cases corresponded to about 12 and 15 grams of milk.)

Table II gives the percentages of the total and diffusible calcium, phosphorus and nitrogen. It is seen that the differences in the amounts of calcium, phosphorus and nitrogen in raw and heated milk, at all temperatures investigated, are within experimental error, which seems to indicate that the diminution in the total calcium of milk on heating which was found by Milroy (7) was due to the filtration.

No significant difference is found at any temperature in the amount of diffusible nitrogen in raw and heated milk, about 4 per cent. of the total nitrogen being always present in the diffusible form.

With regard to the phosphorus, it appears that at higher temperatures, above 175–178° F., about 3½ per cent. of the total phosphorus is changed from the diffusible to the indiffusible state, but at all temperatures below this, the results show no significant differences. It is felt, however, that the experimental error in the method used is too great to permit of any conclusions being drawn from these results and it is hoped to make a further study of the diffusibility of the phosphorus at a later date.

The changes in the diffusibility of the calcium salts are very marked and are shown in Table II.

In milk heated to temperatures varying from 105–120° F. the difference in the amounts of the diffusible calcium was within experimental error.

Duplicate experiments (not given in the Tables) show that while at temperatures of 135–140° F. the amount of the total calcium which becomes indiffusible is only about 0·6 per cent., this increases at 145–150° F. to about 2 per cent., while at temperatures above this the amount becoming indiffusible varies from 2·5 to 3·6 per cent. At all temperatures from 125–209° F. the amount of calcium which had

diffused from the raw milk was definitely greater than that which had diffused from the same milk after heating.

Summary.

Milk which has been heated to temperatures varying from 105–209° F. for half an hour differs from raw milk in its reaction to rennet in all cases.

There is no change in the diffusibility of the nitrogenous substances in milk after heating to temperatures varying from 105–209° F. for half an hour.

Heating to 175° F. and above for half an hour appears to reduce the diffusibility of the phosphorus content of milk.

Heating to 125° F. and above for half an hour causes marked diminution in the diffusibility of the calcium content of milk.

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THE ACTION OF VISCOGEN (CALCIUM SACCHARATE) ON MILK AND CREAM.

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The remarkable property of viscogen (calcium saccharate) of increasing the viscosity of cream, milk, condensed milk, etc. was discovered by Babcock and Russell (1896). These authors showed that in cream which had been pasteurised or heated to high temperatures the clumping or aggregation of fat globules, which characterises the raw product, was destroyed, and the viscosity simultaneously much lowered. Addition of viscogen was found to restore in general both clumping and viscosity. Although viscogen has been in frequent use since, very little information is available as to the mechanism by which it produces these results.

The active part of the substance is known to be the lime, sugar being employed merely as a solvent. Babcock and Russell write: "Regarding the chemical action that is responsible for the formation of these fat groups, we are at a loss to know whether an insoluble phosphate is formed which produces points around which the fat globules cluster, or whether a chemical union takes place between the lime and casein." They attempted to settle the point by trying the effect of adding viscogen to rennet whey. Finding, however, that this treatment did not lead to any increase in the viscosity of the whey (which they argued should contain all the constituents of milk except casein), they concluded that the action of viscogen could not be attributed to the formation of an insoluble phosphate, but was probably connected in some way with the casein.

No further investigation seems to have been made into the matter. Thus, in a recent textbook of Dairy Science (1928) it is stated: "Viscogen, like many protective proteins, greatly enhances aggregation and whipping quality [of cream]. Other factors seem to be involved when this agent is used though no attempt at an explanation has been made."

In the course of some experiments on the effect of acids on cream viscosity, with which the present author was associated, his attention was drawn to the very considerable increases in viscosity produced in rich creams by amounts of precipitated casein which hardly affected

the viscosity of poorer creams. This result might be expected, for it seems probable that the viscosity of a suspension will increase more rapidly than its concentration, and that the higher, therefore, its existing concentration the more rapidly will it respond in viscosity to small additions of solid matter, such as precipitated casein.

It seemed probable, from this point of view, that Babcock and Russell's experiment with rennet whey did not entirely exclude the possibility that precipitated calcium phosphate was responsible for the increased viscosities induced by viscogen in creams. For, a quantity of calcium phosphate which might not affect perceptibly the viscosity of the water-clear serum, might easily cause considerable increases in that of a cream already rich in suspended matter. As, furthermore, Babcock and Russell's viscosity measurements were made with a pipette, their accuracy was hardly sufficient to enable very definite conclusions to be drawn. It was considered of interest therefore to reinvestigate the whole matter.

In the experiments about to be described viscosities were measured by instruments of the Ostwald type. No very elaborate precautions were taken in these measurements, as the object was to use the viscometric method to diagnose the occurrence or otherwise of certain precipitation reactions, and not to record a series of exact determinations for milks or creams of various fat content. For this reason, and as it simplifies presentation, all viscosities are here recorded in terms of the original viscosity of the untreated sample, which for convenience is taken as 100 in every case.

It was recognised, even at the outset of the work, that the action of viscogen could hardly be ascribed solely to a reaction with some one milk constituent such as phosphate. The matter was bound to be more complex. For instance, viscogen is strongly alkaline and is, therefore, likely to affect to some extent the viscosity of the casein in milk through its influence on the hydrogen ion concentration. For a beginning, however, an attempt was made to see whether some or all of its effect could definitely be ascribed to the formation of insoluble calcium phosphate, the significance of its alkalinity and direct influence on casein being considered later as the work developed.

Action of viscogen on phosphates in milk.

Milk serum was prepared by filtering fresh skimmed milk (which contained a little toluene as preservative) through a Chamberland porcelain candle. A second type of serum was obtained from the

precipitation of the casein in skim-milk by acetic acid ("acid serum"). Rennet whey was not used in these experiments as it seemed unlikely to possess any advantages over the Chamberland filtrate.

Artificial creams containing 20 per cent. fat were now prepared by emulsifying butterfat in these sera (to which 0·25 per cent. gelatine had been added previously, as it was found very difficult to secure stable emulsions in the sera alone). The viscosities of sera and creams were determined before and after the addition of just enough viscogen (2 $N/1$) to give a faint pink with phenolphthalein. This is rather more than the quantity recommended by Babcock and Russell in practice, but it was convenient for comparative purposes to work to the definite and not excessive degree of alkalinity shown by this indicator.

Typical results are shown in Table I.

Table I.

Material	Relative viscosity: Initially	Relative viscosity: After addition of viscogen
Chamberland serum	100	112
Acid serum	100	142
20 % "cream" (Chamberland serum)	100	115
20 % "cream" (acid serum) ...	100	146

The values obtained varied considerably with samples of the two sera prepared from different skim-milks, but the general direction of the results was the same in all. The acid sera always showed much greater increases in viscosity than the Chamberland, and their correspondingly heavier phosphate precipitation was very noticeable. This agrees with what is known of the composition of these sera—for the Chamberland filtrate contained only the soluble phosphate, whilst the acid filtrate contained in solution all the inorganic phosphates of the milk whether originally present in soluble, colloidal or insoluble form. The acid serum, notwithstanding its much higher acidity, is believed to correspond more closely to skim-milk in its reactions with viscogen than the Chamberland serum, for it has been shown by Van Slyke and Bosworth (1914) that nearly all the inorganic phosphate in milk (the greater part of which is present as colloidal $CaHPO_4$), and not merely the soluble alone, is capable of forming tri-calcium phosphate on titration with caustic alkali to phenolphthalein. The formation of tri-calcium phosphate in milk will naturally be even more complete if viscogen, *i.e.* essentially calcium hydroxide, is employed for the titration on account of the higher calcium concentration of the solution at the neutralisation point.

This fact is reflected in the higher acidities found for milk when calcium hydroxide is used for titration instead of alkali hydroxide. Thus a sample of skim-milk showed:

Titratable acidity using sodium hydroxide = 16·15 cc. $N/10$ per 100 c.c. milk.

Titratable acidity using calcium hydroxide = 20·3 c.c. $N/10$ per 100 c.c. milk.

It is very probable then that the phosphates in milk will react to viscogen in much the same way as those in acid serum, and they must, therefore, be regarded as a considerable factor in viscogen action.

The synthetic creams used in these experiments resembled very closely in their behaviour to viscogen the sera from which they were prepared. This result was somewhat surprising, as it was expected that the creams, containing as they did much suspended matter, would increase in viscosity to a greater extent than the corresponding sera.

Action of viscogen on casein in milk.

For the purpose of investigating this action, two 40 per cent. creams were prepared, one by emulsifying butterfat in "acid" serum (previously neutralised to about pH 6·2), to which one-ninth of its volume of skim-milk had been added; the other in skim-milk. The second emulsion contained therefore ten times as much casein as the first; in other respects they differed very little. The viscosities of emulsions and continuous phases were determined as before. The results are shown in Table II.

Table II.

	Relative viscosity	
	Initially	After addition of viscogen
(*a*) Acid serum + one-ninth skim-milk	100	152
(*b*) Skim-milk	100	370
40 % "cream" in (*a*)	100	140
40 % "cream" in (*b*)	100	367

Accurate determinations of viscosity were difficult to make after the addition of viscogen, but the general trend of the results is clear.

The presence of much casein greatly increased both the effectiveness of viscogen and the amount of precipitate formed. Thus, the skim-milk appeared to be filled with a fine gelatinous precipitate which showed little inclination to settle, and, to judge from its amount, must have included a considerable quantity of casein.

The presence of 40 per cent. of fat did not seem to influence the

viscosity to any extent—in fact somewhat higher values were obtained in its absence.

It will be remembered that a similar result was obtained for the creams prepared by emulsifying butterfat in milk serum. These results are rather unexpected when it is remembered that Babcock and Russell attributed the action of viscogen ultimately to its power of bringing about clumping in fat globules. The present experiments however were conducted with synthetic creams which may behave differently in this respect. It is worthy of note nevertheless that Babcock and Russell (*loc. cit.*) state: "Under circumstances, which we at present are unable to explain, sometimes the aggregations [of fat globules] are not found in microscopic preparations, although when not evident in this way, the consistency of the treated products is nevertheless greatly increased." The extraordinary increases in viscosity produced by viscogen in skim-milk, which in some samples amounted to six or even eight hundred per cent., seem to indicate that the clumping of the fat globules may not be such an essential factor in its action on cream. It is hoped to deal with this point in a later communication.

To confirm the precipitation of casein by viscogen, two samples of equal quantities of skim-milk, one containing just sufficient viscogen to show a faint pink with phenolphthalein, the other without, were centrifuged for three-quarters of an hour. The lower half of the sample to which viscogen had been added was found after centrifuging to contain a dense gelatinous precipitate whilst the upper half had the ordinary appearance of skim-milk. A measured quantity, drawn off from the top of each sample, was employed for the determination of nitrogen by Kjeldahl's method, with the following results:

Protein remaining in solution in skim-milk containing viscogen, 2·50 per cent.

Protein remaining in solution in skim-milk not containing viscogen, 3·47 per cent.

The addition of viscogen had thus led, in this instance, to a precipitation of about one-third of the casein in the milk. It was now necessary to determine how exactly this precipitation was brought about. There seemed to be two possibilities (*a*) direct precipitation of casein, or calcium caseinate by viscogen, or (*b*) carrying down of casein by the tri-calcium phosphate precipitate. To settle this point it was necessary to work with casein solutions.

Action of viscogen on casein solutions.

A solution of calcium caseinate was prepared by grinding together a mixture of casein and about one-fortieth of its weight of calcium hydroxide, with gradual addition of water. The solution was brought to about pH 6·5 by addition of dilute lactic acid ($N/5$), and filtered through cotton wool to remove any undissolved or precipitated casein. A determination of nitrogen by the Kjeldahl method showed the resulting solution to contain 4·2 per cent. casein. To a portion of the calcium caseinate solution, a small amount of acid potassium phosphate was added, and to another portion an equivalent quantity of di-sodium hydrogen citrate as a blank, so that the titratable acidity and hydrogen ion concentrations of the two should be similar, and that approximately the same quantity of viscogen should be required for each.

In preliminary experiments along these lines, some difficulties were met with, which it will be well to mention in order to explain the reasons for the compositions of the solutions finally chosen for test (Table III). Thus, it was found that the use of much citrate led, as is known to occur in such cases, to the formation of sodium caseinate. As this substance appears to increase in viscosity with increasing alkalinity of the solution, at a different rate from that of calcium caseinate, a solution containing it could not be regarded as furnishing an altogether suitable blank for comparison with the calcium caseinate solution containing phosphate. The difficulty was overcome to some extent by introducing a little calcium chloride into the solution in addition to the sodium citrate, in order to preserve a favourable ratio of calcium to sodium ions, and so reduce the formation of sodium caseinate.

The second difficulty arose with the solution containing acid potassium phosphate. When a little calcium chloride was added to this solution in order to make it similar in all respects (except, of course, the substitution of phosphate for citrate) to that containing citrate an immediate precipitation of casein occurred. Only by careful control of the amount of phosphate present and of the hydrogen ion concentration of the mixture could calcium salt be added without causing precipitation.

It was found best to add a combined solution containing a phosphate mixture of pH 6·2 and calcium chloride. By this treatment no precipitation of casein took place if the phosphate and calcium concentrations did not exceed those normally present in soluble form in milk serum.

Addition of viscogen to the caseinate solution containing phosphate was found to give considerable increases in viscosity comparable to

those obtained with skim-milk. The casein solution which did not contain phosphate showed only slight increases in viscosity, probably attributable to its increased alkalinity.

Table III.

Solution	Relative viscosity: Initially	Relative viscosity: After addition of viscogen
4·2 % calcium caseinate containing $M/50$ phosphate and $M/80$ $CaCl_2$	100	423
4·2 % calcium caseinate containing $M/50$ Na_2H Citr. and $M/80$ $CaCl_2$	100	124

It is evident from these results (Table III) that viscogen has little direct effect on calcium caseinate, and that the precipitate of caseinate which occurs when it is added to milk or cream is due to its being flocculated or carried down by the freshly precipitated calcium phosphate. That the alkaline earth caseinates have the property of being flocculated in this manner was remarked by Osborne (1901) who states "They are precipitated from their solution by addition of any finely divided substance; connected with this is their inability to pass through the pores of a clay filter." The alkali caseinates, on the other hand, are not so precipitated.

We have here an explanation of the following observation which seemed, in the early stages of this work, to raise difficulties to the acceptance of any kind of phosphate precipitation as the central reaction between viscogen and milk. Alkali hydroxide is found to increase the viscosity of skim-milk somewhat, but not nearly to the same degree as viscogen. From the work of Van Slyke and Bosworth (*loc. cit.*) it is probable that tri-calcium phosphate is largely precipitated by both reagents, but no doubt to a somewhat greater extent by the viscogen. Nevertheless viscogen appears to be superior to alkali in increasing viscosity to a far greater degree than the slightly heavier phosphate precipitate obtained by its use would seem to warrant. The co-precipitation of casein, noticed later, and Osborne's observations on the alkali and alkaline earth caseinates make the reason for this difference clear. Alkali hydroxide precipitates tri-calcium phosphate, but on the other hand converts calcium caseinate into the alkali caseinate (as is obvious from the greater translucency of the milk), so that co-precipitation of caseinate does not occur when it is used.

INFLUENCE OF THE ALKALINITY OF VISCOGEN.

The alkali and alkaline earth caseinates show marked variation in viscosity with change of hydrogen ion concentration. Loeb (1922) working with 1 per cent. barium caseinate over the range *p*H 6–12 found a maximum viscosity at about *p*H 11·5. Between the *p*H values 6·5 and 8·5 (around which the phenolphthalein change becomes visible) the increase in viscosity amounted to 6 per cent. Zoller (1920) using more concentrated solutions of casein (9 per cent.) in various alkalies and alkaline salts found the maximum viscosity to occur around *p*H 9·2. The alkaline earth caseinates were not examined, but lithium caseinate, which probably resembled them, showed a 25 per cent. increase in viscosity between *p*H 6·6 and 8·5. From these results it appears unlikely that the viscosity of milk (about 3 per cent. casein) will be greatly increased by the change of reaction due to viscogen. The determination cannot be made directly with milk on account of the other reactions which occur. A 3 per cent. solution of calcium caseinate however, originally of *p*H 6·5, showed a 6·5 per cent. increase in viscosity on addition of sufficient viscogen to produce a perceptible tint in phenolphthalein. This increase is seen to be small compared with those produced by the other reactions of viscogen. The action of this agent may thus be regarded as almost entirely due to the formation of a precipitate of calcium phosphate, which carries down considerable quantities of casein.

A number of observations of various authors on the behaviour of milk and cream to viscogen can readily be explained from this point of view.

Thus Babcock and Russell (*loc. cit.*) noticed that the consistency of milk treated with viscogen was intimately related to the development of acidity, and that where the acidity had previously been artificially increased by the addition of any acid, the consistency was much greater.

The reason for this is clear. The higher acidity necessitates the use of more than the usual amount of viscogen for partial or complete neutralisation, so that more calcium is available for the production of tri-calcium phosphate[1], and heavier precipitation of the substance can occur with correspondingly heavier co-precipitation of casein.

Again Hammer (1916) observed that the addition of viscogen to milk brought about the formation of an extremely deep cream layer, often extending nearly to the bottom of the creaming cylinder. This has

[1] Precipitation of tri-calcium phosphate in milk is limited by shortage of Ca rather than P. This substance requires a Ca/P ratio 1·94 whereas in milk the ratio $\frac{\text{inorganic Ca}}{\text{inorganic P}}$ is usually about 1·25.

been taken to mean that viscogen leads to the formation of large irregular clumps of fat globules, which do not pack closely together.

It seems more probable from what has been shown here of the nature of viscogen action that clumping is not really concerned, and that the very deep cream line is due to the difficulty with which the phosphate-casein precipitate is "floated" by the entangled fat globules.

SUMMARY.

The mechanism by which viscogen (calcium saccharate) brings about an increase in the viscosities of milk and cream has been investigated.

The primary reaction appears to be the formation of a precipitate of insoluble (tri-calcium) phosphate. Considerable quantities of casein are carried down by the precipitate, and this co-precipitation of casein is probably the single factor which most influences the viscosity.

Casein is not directly precipitated by viscogen, but the viscosity of its solutions is slightly increased as a result of their higher alkalinity due to this reagent. This action of viscogen is relatively unimportant in influencing the viscosity of milk or cream.

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INVESTIGATIONS ON YIELD IN CEREALS. VI.

A. A DEVELOPMENTAL STUDY OF THE INFLUENCE OF NITROGENOUS TOP-DRESSING ON WHEAT.

B. A MEASUREMENT OF THE INFLUENCE OF DISEASE ("TAKE-ALL") UPON THE YIELD OF WHEAT.

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(With Two Text-figures.)

CONTENTS.

GENERAL INTRODUCTION.

This series of investigations embraces studies of the more important factors, external and internal, which govern yield in field crops of cereals. It has so far dealt with the causes and influence on yield of fluctuations in density of plant population and also with certain inter-field differences of environment. Throughout, an analytical method has been employed, in which periodic observations have been made upon small samples distributed over a representative acre in a field crop. The best size of sample has proved to be a one-foot length of row, *i.e.* row of plants as seeded by the drill. The basis of this method—a "census" of an acre of corn—has been described and critically examined in an earlier paper (Engledow (1)). Analyses of certain external factors affecting yield have been recorded by Engledow (2) and by Doughty and Engledow (3).

Among the external yield factors top-dressing with a nitrogenous

fertiliser has recently acquired special importance. It is a controllable factor and, while involving little outlay, brings, in some winter corn crops, a handsome yield increase. There is an abundance of practical experience to vouch for this increase but a lack of reliable experimental records. That extremely reliable estimates of increase are possible has been shown, however, by the comprehensive investigation of Eden and Fisher (4). Uncertainty still surrounds the question of the optimum time of application of nitrogenous top-dressings for winter corn. In practice the condition of the soil, particularly on heavy land, largely determines the time of application. But, aside from this, farming opinion as to best time of application is variable. Dressings are sometimes applied to winter corn as late as the end of March; a growing body of opinion favours mid-February or even earlier. It is patent that biologically optimum time of application can be determined only by studies of development of field crops.

Developmental studies, made in the earlier investigations of this series, have shown that a critical period occurs in the growth of field crops of wheat usually during the month of March. This period has two striking characteristics. It marks the time up to which tillering is unaffected by spacing. That is to say, up to perhaps the end of March all the widely fluctuating densities of population occurring from point to point among field plants show a common degree of tillering. Onwards from this critical period a definite negative correlation asserts itself between population density and tillering. Up to the critical period, therefore, conditions other than spacing are the determiners of tillering. Among these conditions are soil fertility, drainage, and, no doubt, temperature.

In the second place, the critical period marks the time before which are formed the tillers destined to bear ears at harvest. As a fact it is impossible to determine time of formation of a tiller with precision. For simplicity the time at which a tiller first becomes visible is recorded as the time of formation. In typical field crops of wheat in the Eastern Counties tillering may continue well on into June. Considerably before it ceases some tillers commence to wither. It is estimated that of all the tillers visible in mid-May only about 40 per cent. survive and bear ears at harvest. These survivors are, in general, the tillers formed before the critical period.

If a top-dressing is to increase yield by augmenting the average number of ears per plant, it must be available to the plant before the critical period of tiller formation. It is possible, of course, that a nitro-

genous dressing may somewhat defer the critical period. Further, an application too late to affect ear-formation may increase yield by its influence on ear-size. Finally a connection is invariably found between ear-production and ear-size.

These considerations show the importance of developmental studies of fertiliser action and it was in the light of them that the present investigation was undertaken. Shortly after ear-emergence it became evident that the field was heavily infected with a group of diseases loosely called by farmers "take-all" or "whiteheads." Examination of the census samples was so arranged as to afford a measure of the incidence of the diseases and of their effects on yield.

PART A. A DEVELOPMENTAL STUDY OF THE INFLUENCE OF NITROGENOUS TOP-DRESSING ON WHEAT.

I. Experimental procedure.

The field used for the experiment was selected as being likely to show well-defined response to nitrogenous top-dressing. It was on gault clay and therefore imperfectly drained. Moreover it had carried a crop of wheat in the preceding year. Cambridge Browick wheat at 2½ bushels per acre was drilled on Nov. 7, 1927. On Feb. 28, 1928, sulphate of ammonia at a rate of 1½ cwt. per acre was applied by distributor to four long, narrow strips suitably arranged with four undressed (control) strips of the same size. The field was permanently thrown up into wide "lands" or ridges, separated by water furrows. Corn drilling was across these ridges. The size and arrangement of the eight strips were so chosen as to harmonise with the width of the ridges and to afford comparisons of dressing and no-dressing both across a ridge and across a furrow.

On every strip 25 samples were marked out. A sample was a one-foot length of corn drill-row. Appendix I fully describes the lay-out of strips and distribution of samples. Between germination and early April six counts were made on the marked samples to determine number of plants per foot and number of tillers per plant. After early April accurate counting necessitated uprooting the plants. Two later counts, one in April and one in May, were carried out by uprooting samples at a suitable remove from the marked samples. At harvest every marked sample was uprooted, the plants and the ears on every plant counted, and then the grain from the combined plants of the sample weighed. In addition 75 extra samples were lifted on every strip. These were situate at fixed distances from the marked samples. On every one of

them a count was made of plants (per foot) and tillers (per plant). The 75 samples from every strip were then bulked, threshed, and weighed. In what follows the originally marked 25 samples will be called "marked" samples: the additional 75 harvest samples will be called "extra" samples. Reliability of result is dealt with in Appendix II.

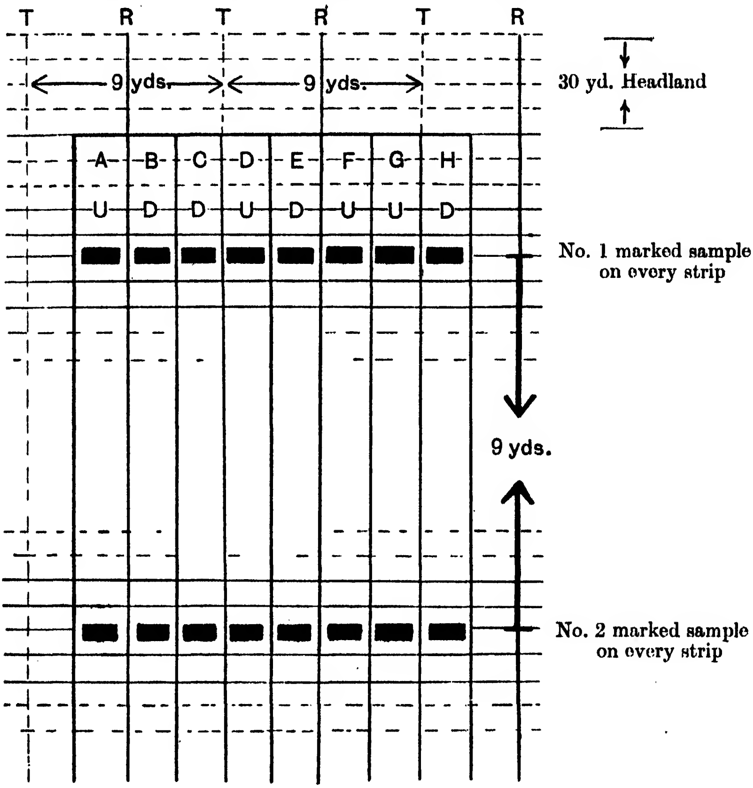

Diagram 1. Showing lay-out of strips and distribution of samples. (For full details see Appendix I.)

II. An outline of crop development.

It is now recognised that in cereal plants tillering is a valuable index to general development. This is especially true in the first half of crop life. It is the practical simplicity of this index which gives it importance in the study of crops under field conditions. But the index-value of tillering can be realised only from periodic records throughout life. In this experiment records were made from three months after sowing—when the first side tillers were seen—up to early May. After this latter

date counting became difficult by reason of the abundance of small side tillers. Moreover, May-formed tillers invariably die without maturing. Table I, a résumé of plant population and tillering data, affords a conspectus of the stages of crop life. It contains average values based on four combined dressed strips (*i.e.* 100 samples) and four combined untreated ones.

Table I. *A résumé of plant development on the dressed* (D.) *and untreated* (U.) *strips* (*mean values for the four strips of each kind*).

Count No.		Date	Untreated U.	Dressed D.
	Sowing	Nov. 7	—	—
1	Av. no. of plants per foot	Feb. 7	14·6	14·0
2	Av. no. of plants per foot	Feb. 20	13·8	14·7
	Av. no. of side tillers per plant		0·20	0·19
	Sulphate of ammonia applied	Feb. 28	nil	1½ cwt per acre
3	Av. no. of plants per foot	Mar. 7	13·2	13·4
	Av. no. of side tillers per plant		0·36	0·40
4	Av. no. of plants per foot	Mar. 14	13·6	13·8
	Av. no. of side tillers per plant		0·60	0·51
5	Av. no. of plants per foot	Mar. 21	13·4	13·6
	Av. no. of side tillers per plant		0·78	0·74
	Effect of dressing clearly apparent in foliage colour of D.	Apr. 4	—	—
6	Av. no. of plants per foot	Apr. 4	12·8	13·1
	Av. no. of side tillers per plant		1·45	1·32
	Correlation: plants per foot and no. of side tillers per plant		−0·11	−0·36
7	Av. no. of plants per foot	Apr. 18	13·4	14·1
	Av. no. of side tillers per plant		1·73	2·24
	Correlation: plants per foot and no. of side tillers per plant		−0·45	−0·48
8	Av. no. of plants per foot	May 5	13·6	14·2
	Av. no. of side tillers per plant		1·62	2·14
	Correlation: plants per foot and no. of side tillers per plant		−0·40	−0·63
9 (harvest)	Av. no. of plants per foot	Aug. 11	10·4	9·9
	Av. no. of ears per plant		1·06	1·16
	Rate of yield per acre (bushels)		19·1	26·4
	Av. yield per foot of row (gm.)		8·38	11·58
	Av. yield per plant (gm.)		0·81	1·17
	Av. yield per ear (gm.)		0·76	1·00

Sowing, on Nov. 7, 1927, was carried out very successfully on a tilth which, for heavy land and with wheat after wheat, was highly creditable. This is reflected in the data of the first count on Feb. 7, 1928. The mean numbers of plants per foot were U. = 14·6 and D. = 14·0 with corresponding coefficients of variation (100 σ/M) of U. = 39·5 per cent., D. = 40·5 per cent. These coefficients are below the average for heavy land wheat.

Population density, defined as average number of plants per foot of drill-row, calls for only brief notice. It displayed marked localised fluctuation which gave rise to corresponding fluctuation in rate of yield per foot length of row. For the present, however, these aspects of development are not of intrinsic interest. The general considerations arising from them have been fully examined in earlier publications. It is apparent from Table I that on both dressed and untreated strips plant population remained at about constant strength up to early May (8th count). Subsequently, heavy casualties occurred, with the result that instead of about fourteen plants per foot, as in May, there were at harvest only about ten. So heavy a mortality has not previously been recorded in field studies. The explanation is believed to lie in an unusually severe attack of what farmers loosely call "take-all," or "whiteheads," which usually, as a fact, represents a number of fungi. Full attention is given to this matter in Part B (*infra*).

At no count was there a statistically significant difference in mean population density between the dressed and untreated strips. It is thus evident that increased survival rate is not induced by a February nitrogenous top-dressing. In the Eastern Counties winter wheat commonly shows a survival rate of the order of 70–80 per cent. Rarely, the survival may fall to 50 per cent. or even lower. Thus on certain exposed and wet fields in the winter of 1926–7, where during prolonged frost the snow was blown away from the plants, losses of even 75 per cent. were occasioned. But generally, in corn, adverse winter influences make their mark not on plant survival but on tiller production and, later, on the development of those tillers destined to form ears. It is, therefore, to stimulation of these tillers that the policy of nitrogenous top-dressing should be directed.

Roughly speaking the commencement of tillering was between the first and second counts, *i.e.* in mid-February. This is late for an early-November sowing, a fact explained by the cold, heavy soil, and by the low temperatures of December and January. Subsequent progress is shown in Diagram 2 for which the values have been extracted from Table I. The first statistically significant difference in tillering between U. and D. occurred on April 18. That at the three counts preceding this the U. value was greater than the D. value is no more than coincidence. It seems then that the effect of top-dressing on tillering first manifested itself between April 4 and April 18. For simplicity April 10 may be regarded as the day on which tillering-response became apparent. The first evidence of the effect of nitrogenous top-dressing on corn is

always increased leaf area and a deeper green colour. In this case the dressed strips were observably deeper in colour on April 4, *i.e.* about a week before they showed increase in tillering over the untreated strips. There appears to be no clear evidence as to the time elapsing between the application of any specific top-dressing and the commencement of

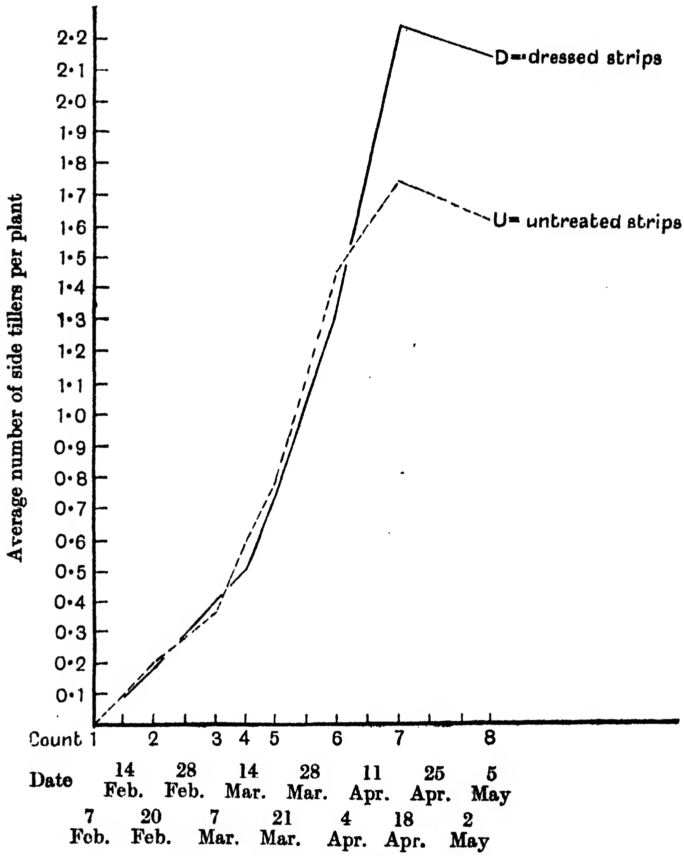

Diagram 2. The progress of tillering.

its influence upon growth. For nitrogenous dressings on corn, which are used as stimulants, an estimate of this time is essential to the determination of optimum period of application. Soil and weather factors would naturally influence its duration. In this experiment relatively little rain fell between the application of the fertiliser and the third week in March. It is possible, too, that temperature remained for some time the limiting factor to tiller-formation.

Rate of progress of tillering is shown in Diagram 2. Between the 1st and 8th counts—the period Feb. 7 to May 5—there appear to have been four phases of tillering activity for both the dressed (D.) and untreated (U.) strips. Up to March 7 progress was relatively slow. During the next three weeks (count 3 to count 6) it was much more rapid. After this time a striking difference appeared between U. and D. In the untreated (U.) crop, rate of tiller increase was lower than during the previous month: in the dressed (D.) crop it was even more rapid than before. It is therefore evident that the influence of the top-dressing upon tillering became apparent in the third phase of development, *i.e.* roughly, in the first half of April. The fourth phase—April 18 to May 5—is one of decline in average number of side tillers per plant. Recorded observational notes show that it was early in this period that dead or definitely dying tillers were first seen. The possible influence of disease on early tiller mortality is discussed in Part B.

Correlation between number of plants per foot and average number of tillers per plant may next be noticed. On D. it first attained statistical significance at the 6th count (April 4); on U. significance was not recorded until a fortnight later. Now it was in the third tillering phase (roughly count 6 to count 7) that rate of tiller production was sharply accelerated on D. but began to show retardation on U. The differences between U. and D. in tillering rate and spatial correlation suggest that, up to early April, available soil nitrogen was the limiting factor to tillering. On the U. strip it so remained until April 18 (7th count: $r = -0{\cdot}45$), when spacing became limiting. But on the D. strips available nitrogen ceased to be limiting in early April and the spatial factor became effective (April 4: 6th count: $r_{\mathrm{U.}} = -0{\cdot}11$ (non-significant): $r_{\mathrm{D.}} = -0{\cdot}36$).

This outline of tillering progress, and the suggested phases, are necessarily imperfect representations of actual development. A true picture could be delineated only by very frequent counts on great numbers of samples. Even then the detail would be obscured. It is not practicable to record tillers save as whole units; but, developmentally, a very small tiller signifies less than a large one though both, statistically, must be recorded as one tiller. Moreover, in the final phase (count 7 to count 8) loss and increase of tillers both occur, the recorded numbers showing merely the difference between the two processes. In spite of these defects the developmental record is such as to establish the existence of time phases in tiller formation. That these phases are of essential importance in relation to time of top-dressing is

apparent from the critical period hypothesis outlined in § I (*supra*). How tillering phases are related to temperature and other factors of environment is not determinable from the available data. The relationship must be highly complex. It is partly governed by the nature of the tillering process. Side tillers arise not only from the main axis but from the side tillers of that axis, in turn, and from the side tillers of these. A tillered plant is biologically not a unit but an aggregate of units (tillers) differing in chronological and physiological age. Thus the influence of any weather factor on a single plant is the sum of its influences upon an aggregate of units of growth.

In § I (*supra*) attention was directed to a "critical period" of growth which for winter corn falls usually towards the end of March. Up to this period spacing, within the spatial limits of field crops, exercises no influence on tillering. Moreover only those tillers which appear before the critical period form ears at harvest. Now in this experiment the top-dressing induced no tillering increase—as compared with the untreated crop—until April 10. Accordingly on the critical period hypothesis it should have produced no increase in number of ears per plant at harvest. It is interesting to note from the data of the ninth (harvest) count that, actually, the superiority in ear-formation on the dressed strips amounts to barely 10 per cent. (average number of ears per plant, U. = 1·06: D. = 1·16). Ear-formation was, in this experiment, unusually low in relation to early tillering. Indeed, as a whole, the plants on both dressed and untreated strips produced little more than a main axis ear, the side tillers being sterile. Disease (*vide* Part B *infra*) was undoubtedly the cause of this. But it is important to note that top-dressing, producing no tillering superiority before what is normally the critical period of tillering in winter wheat, brought about only a very small superiority in average number of ears per plant at harvest. For practical purposes it may be assumed, then, that a late-February top-dressing did nothing to augment yield by increasing ear-formation.

III. Top-dressing and yield.

Over the whole field, including the experimental strips, the yield was abnormally low for the type of land and level of husbandry. The major responsible causes were two wheat crops in succession and the heavy "take-all" attack. That top-dressing produced a marked effect on yield is evident from the data of the ninth (harvest) count, Table I. The yields were at the rate of 26·4 bushels per acre on the dressed strips (D.) and 19·1 on the untreated (U.)—a difference of 38 per cent. This is equivalent

to a difference of £1. 17*s.* per acre which, after deducting 17*s.* per acre for top-dressing (labour and material), leaves a net profit increase of £1 per acre.

The analytical basis of this increase is readily shown by expressing certain plant attributes of the dressed strips (D.) in terms of those of the untreated (U. = 100). Almost the whole effect of top-dressing on yield

	Yield per acre	Yield per plant	Average no of ears per plant	Average yield per ear
D./U.	138·2	144·4	109·4	131·6

per acre arose from its effect on ear-size. That by treatment and also by uncontrollable environment ear-production and ear-size may, within limits, be independently influenced accords with our general knowledge of cereal growth. A modification of practice in nitrogenous top-dressing is clearly suggested by the results which have been described. Common practice is a "spring" dressing which is usually applied at some time during February or March. Such a dressing augments ear-size but cannot raise yield per acre in any other way. For increased ear-formation an earlier dressing would be necessary—possibly in December. On heavy land the distributor can rarely work during the three months November–January. Possibly, therefore, the most practicable procedure would be a half-dressing at sowing time and another at the beginning of February. This proposal would naturally have to be examined in the light of certain well-known circumstances. Excessive autumn growth may lead to that serious but little understood condition known as "winter proud." On some soils the autumn dressing might be "washed down" below root range. It is, again, manifestly possible that early tillering and winter growth generally are primarily limited by temperature. An investigation, on a census of an acre basis, is being made upon the influence of time of application of a nitrogenous fertiliser.

PART B. A MEASUREMENT OF THE INFLUENCE OF DISEASE ("TAKE-ALL") UPON THE YIELD OF WHEAT.

It is commonly assumed that disease plays an unimportant part in English cereal production. Total loss of crop is very rare but evidence of relatively serious damage is every year apparent. Imperfectly filled grain is a very common blemish in our wheat. This, while usually dismissed as a result of "bad weather," arises in most cases from yellow rust. The persistence of bunted wheat on the market is no less regrettable than it is inexcusable. Interest in prevention and the

production of resistant varieties can be stimulated only by definite ascertainment of loss. This is impracticable with rust but by no means difficult with "take-all" or barley leaf-stripe. The "census of an acre" method, employed in the main experiment, lends itself to measurement of loss from disease. In the crop on which the investigation of Part A was made, an unusually heavy outbreak of "take-all" occurred. The observations connected with top-dressing effects were therefore extended to afford a measure of the influence of this disease.

"Take-all" or "whiteheads" has been known in England since 1861. It is believed to have gained ground somewhat rapidly in the Eastern Counties during the past decade. In field crops, as in this experiment, the true "take-all" pathogene (*Ophiobolus cariceti* (Berk. and Br.) Sacc.) is usually associated with certain other fungi. Species of *Leptosphaeria* and *Fusarium* are often found. The extensive "stem rot" and "foot rot" investigations recently made in North America indicate the difficulty of diagnosing the numerous pathogenes of the association in which *Ophiobolus* frequently appears. In these passages, therefore, "take-all" has the popular agricultural application and refers to a somewhat uncertain combination of fungi.

A measurement of "take-all" damage was made on every strip (*i.e.* 4 untreated = U. strips and 4 dressed = D. strips). For this the extra 75 harvest samples per strip were used. The total ears from the 75 samples were classified as healthy and affected, the total grain from each class being then weighed. Affected ears were readily distinguishable. The whole of their grain was completely shrivelled and the chaff discoloured by a secondary attack of *Cladosporium herbarum*.

Disease was not suspected in early crop life although premature tiller mortality was probably symptomatic. Some three weeks after ear-emergence, "take-all" suddenly became apparent. A considerable proportion of the ears swiftly assumed the familiar "bleached" colour. Developmental records afford a picture of the course of the disease. Actual death of plants was a striking feature. Up to the beginning of May there were no signs of mortality and Table I indicates constancy of population at about 14 plants per foot from February to May. But at harvest there were only 10 plants per foot or 70 per cent. of the May value. In numerous investigations virtual constancy of population from May to harvest has been shown to be normal. One emphatic effect of "take-all" was thus 30 per cent. loss of plant in the latter half of crop life.

Tillering was probably affected at an early period. Diagram 2 shows that average number of side tillers per plant decreased on both

dressed (D.) and untreated (U.) strips between the 7th and 8th counts (April 18 and May 5). It was, as a fact, in making the 7th count that tiller mortality was first clearly observed. Normally, of the tillers present in May a high proportion dies, and it is impossible to separate normal mortality from tiller loss induced by "take-all." Tillering reached its maximum at the 7th count (April 18) and of the total stems then present the proportions which survived to form ears were for U., 38 per cent. and for D., 35 per cent. Proportions of this order might characterise crops free from "take-all." It therefore seems probable that while the disease hastened tiller mortality it did not augment it.

Table II. *The ear-damage induced by "take-all" on the four combined untreated* (U.) *strips and the four combined dressed* (D.) *strips.*

	U.	D.
Percentage of total ears affected	32·5	24·6
Av. yield of grain per ear (gm.) for all ears	0·76	1·00
,, ,, healthy ears	0·98	1·21
,, ,, affected ears	0·27	0·39

The final manifestation of damage was complete abortion of all grains in affected ears. Table II shows the incidence of ear-damage. Diseased grain was worthless even as tail corn so that ear-damage must be taken as a measure of loss of potential yield. Loss of the order of 25–30 per cent. is fortunately rare in this country but the results indicate the dangers to be apprehended from the "take-all" group of fungi.

There are indications of difference between disease effects on the dressed and the untreated strips. Ear-damage was higher on the untreated crop. Moreover the ratio of yield from affected and from healthy ears was for U., 0·274 and for D., 0·322. Conjointly, these facts suggest that top-dressing, by early stimulation of potentially ear-bearing tillers, definitely tended to reduce loss from disease. It is interesting to note that the 38 per cent. yield increase attributable to top-dressing more than counterbalanced the loss from disease.

APPENDIX I. STRIP LAY-OUT AND DISTRIBUTION OF SAMPLES.

The field was permanently thrown up into broad lands separated by water furrows with the surface of the ground arched or cambered from furrow to furrow in the manner familiar on heavy land. Corn drilling was at right angles to the furrows and the experimental strips in the direction of the furrows. From furrow to furrow (T, T in Diagram 1) was 9 yards. The strips were made 6·75 feet wide so that a "land" accommodated four strips. This strip width was further convenient in that it

allowed a strip to be dressed with fertiliser by a single journey of the manure distributor. Eight strips, *A–H*, were used, four (*ADFG*) receiving no fertiliser and four (*BCEH*) being given a dressing of sulphate of ammonia at the rate of 1½ cwt. per acre. In Diagram 1 an untreated strip is designated U. and a dressed one D. The incidence of U. and D. in relation to the furrows is shown by Diagram 1, in which *T* shows the line of a furrow and *R* of a ridge (*i.e.* the crest of a land). By means of this arrangement two U.–D. comparisons were afforded across a ridge, viz. strips *A–B* and *E–F*, and two across a furrow, viz. strips *C–D* and *G–H*. Other comparisons may be made, as for example U. on the high and low portions of a land.

Twenty-five samples, each a one-foot length of corn drill-row, were marked at regular intervals on every strip. These lay on a line parallel to the furrows and at a distance of one foot from the median axis of the strip. This distance ensured that the samples were out of the horse-walk when the distributor was used. Further, the samples were at sufficient distance from the margins of strips to escape possible irregularities in fertiliser distribution. A simple procedure was adopted in determining the positions of the samples. On every strip a cord was pegged down at a distance of one foot from the median axis of the strip. Then on strip *A* a foot-length, sample No. 1, was marked by two short pegs on a corn drill-row distant about 3 yards from the end of the strip. One peg was inserted next to the cord, the other at a measured distance of 1 foot from it. A distance of about 9 yards was then paced from sample No. 1 down the strip. On the corn drill-row which lay nearest to the end of the ninth pace sample No. 2 was similarly marked by pegs. In this manner 25 samples were marked on strip *A*. Samples were similarly marked on the other strips, the same corn drill-rows being used as were selected for strip *A* (see Diagram 1). The 9-yard (paced) interval between samples allowed space for drawing extra samples and ensured that samples were drawn at random from the work of the separate coulters of the corn drill.

The samples for the two counts in late April and May had to be uprooted. They were taken at distances down the strip of 1 yard and 2 yards respectively from the 25 marked samples. At harvest three extra samples were drawn at distances down the strip of 3, 5 and 7 yards from every marked sample. Manure distributors are faulty implements and there is considerable difficulty in ensuring specified applications on marked strips. Mr F. Rayns, Director of the Norfolk Agricultural Station, has, however, succeeded in overcoming this difficulty by "trial

runs" of the distributor before commencing on the experimental area. The fertiliser is weighed into and out of the distributor at every run and thus, by trial, an accurate setting obtained. A further difficulty lies in irregularity of distribution in the first few yards of movement of the distributor. To meet this, in the experiment, a 30-yard headland was left at the end of the strip (Diagram 1). Distribution proved to become uniform during the movement of the distributor across the headland to the edge of the strips.

Harvesting of the samples (25 + 75 per strip) offered no difficulties and was carried out without damage to the general crop immediately before the binder came into the field.

APPENDIX II. ON PROBABILITY OF RESULT AND SOME CONNECTED QUESTIONS.

Estimates of tillering and development offer far greater difficulty than those of simple yield per unit area. The underlying reasons, fully discussed in later passages, lie essentially in the experimental necessity of very small samples of crop for tiller counts. To connect the stages of development with final yield it is desirable to determine yield from the same small samples of crop as are used to study tillering. Thus the yield estimations in which development studies culminate suffer a limitation from which variety, manurial, and other simple "yield trials" are free. This may be removed by drawing samples additional to and larger than those used in tiller counts. In practice such an extension is not easy because of the considerable amount of work already involved in counting and weighing the normal developmental samples.

In this experiment sampling for yield was extended by drawing three extra samples at definite distances from every one of the 25 permanent samples on every strip. On the one hundred (25 + 75) samples thus taken from every strip, the number of plants and the number of ears on every plant were counted. For yield, every permanent sample (25 per strip) was separately weighed: the extra samples (75) on every strip were bulked, threshed, and weighed.

In the many census studies made in this series of investigations the coefficient of variation ($V = 100\,\sigma/M$) has had the following ranges of values:

For number of plants per foot at all stages, including maturity	$V = 30$–50 per cent.
For average number of side tillers per plant (up to May) on separate one-foot lengths ...	$V = 30$–50 ,,

For average number of ears per plant at harvest on separate one-foot lengths ... $V = 20$–30 per cent.
For yield per foot length of row ... ... $V = 35$–50 „

Values within these ranges were found for the eight strips on which the present experiment was based.

At all stages of development reliability of result was first gauged by uniting the data from the 4 D. (dressed) and from the 4 U. (untreated) strips. The standard deviation of the difference between two means, each based on $4 \times 25 = 100$ samples, or for harvest data $4 \times 100 = 400$ samples, was then determined. Judged thus, all the differences employed in the body of the text proved to be clearly significant. As a further test, adjoining U. and D. strips were compared. Diagram 1 shows the appropriate pairs to be: *B–A*, *C–D*, *D–E*, *E–F*, *H–G*.

In average number of plants per foot at pre-harvest counts no significant differences were found either between the combined 4 D. and the combined 4 U. strips or in any of the strip-pair comparisons. The harvest data displayed one exception, for average population density on strip *C* was just significantly less than on the adjoining strip *D*. As shown later, the *C–D* strip pair was similarly exceptional in average yield. Observations on the growing crop exposed the probable cause of this exceptional behaviour. When the effects of top dressing became manifest in foliage and general growth, about one-third of strip *C*, at the end where the distributor was first led on to the experimental area, remained unaffected. On the remainder of strip *C* the effects of the fertiliser were patent. This effect persisted and was reflected in the grain yields of the samples from the one end as compared with those from the rest of the strip. It is evident that the distributor was working very imperfectly on about one-third of the length of strip *C*.

Tiller and ear-formation may be treated in two ways. The average number of tillers (or ears) per plant may be calculated for every sample foot-length and then the mean of these sample-averages be found. This will be called the "per-foot" method. Alternatively, the total number of tillers on all the samples of a strip may be divided by the total number of plants—the "per-plant" method. The standard deviation is naturally higher for the per-plant method, but the number of observations is greater, and, as calculation shows, the margin of significance is lower than for the per-foot method. The relation between the two methods is discussed in a later passage. All tiller and ear-formation differences between D. and U. have been tested for significance by both methods. The results of tests by the per-foot method only will at present be considered.

Significant differences in tillering between U. and D. first appeared at the 7th count (Table I, $M_{D.} = 2{\cdot}24$; $M_{U.} = 1{\cdot}73$). Of the five inter-strip comparisons only three displayed significant difference. It is noteworthy, however, that all five were of the same sign, viz. D. − U. = +. Thus the significant difference displayed between the combined 4 D. and the combined 4 U. strips appears to be acceptable. At the 8th count, the last before harvest, the D.–U. tillering difference was definitely significant.

Ear-formation, as average number of ears per plant at harvest, was estimated from 100 samples, or about 1000 plants, per strip. The differences between adjoining dressed and untreated strips were:

$$B{-}A = 0{\cdot}140;\ C{-}D = 0{\cdot}083;\ E{-}D = 0{\cdot}121;\ E{-}F = 0{\cdot}081;\ H{-}G = 0{\cdot}159.$$

The limit of significance (as $3\epsilon_{U.D.}$) was in all these cases 0·070 or less. Thus the small U.–D. difference in ear-formation (Table I, $M_{D.} = 1{\cdot}16$; $M_{U.} = 1{\cdot}06$ for 400 samples each) was clearly significant.

Average yield per foot was estimated from 100 samples per strip but the relevant standard deviation from only the 25 permanent samples. Of the five inter-strip differences only C–D failed to exceed the limit $3\epsilon_{U.D.}$ ($\epsilon_{U.D.}$ = standard deviation of difference between two means each based on 100 values). A probable reason for this exception has already been suggested. It may be noticed, however, that even the C–D difference exceeds the limit $3\epsilon_{U.D.} \times 0{\cdot}6745$. The differences, followed by three times their appropriate probable errors, were:

$$B{-}A = 2{\cdot}72\ (1{\cdot}23);\ C{-}D = 1{\cdot}89\ (1{\cdot}70);\ E{-}D = 4{\cdot}43\ (1{\cdot}34);$$
$$E{-}F = 4{\cdot}93\ (1{\cdot}24);\ H{-}B = 3{\cdot}15\ (1{\cdot}34).$$

The average yield difference between the combined 4 D. and the combined 4 U. strips (*vide* Table I) was 38 per cent. and from the above tests the significance of this appears certain.

Tiller counting, and the use of short sample lengths of drill row, involve some important considerations. It is impossible in practice to discriminate between a side tiller 2 mm. long and an older one of 2 or 3 cm. length. Each must be recorded as one tiller. Thus from a biological point of view tiller counting involves a "grouping" of stages of development. How this affects measurement of dispersion cannot, of course, be determined. One result of grouping is to give to the frequency distribution of number of tillers per plant, in early growth, an extreme asymmetry which, later, is continuously reduced.

The necessity for short sample lengths of drill row is twofold. Considerable lengths, *e.g.* 12 feet of row, would often give a very imperfect

measure of spatial interval. For example, such a length might have 24 plants in the first 3 feet, none in the next foot, 48 in the next 2 feet, and so on. Within limits, the shorter the sample the more accurate the estimate of spatial interval. But a certain "end error" is inevitable, as one end of a measured length may coincide with a plant. The inclusion or exclusion of such a plant is arbitrary. End-error would be serious with a 2-inch sample. After many trials, a length of 1 foot of drill row has been adopted in all the investigations of this series. Another and compelling reason for short samples arises from the arduous nature of tiller counts. Long samples would necessarily involve relatively few samples.

It is desirable to contrast investigations on tillering with simple measurements of the final attribute yield which they help to interpret. In yield trials, plots of $\frac{1}{50}$ acre may be replicated to give high significance without involving great labour. But a single $\frac{1}{50}$ acre plot contains over 1300 foot-lengths of drill row. Tillering can be determined only by counting individual plants; and to carry out counts on more than a few hundred sample lengths would be impracticable. It is thus extremely difficult to bring into tiller studies the reliability now readily procurable in yield trials.

The necessity of employing few and scattered small samples instead of specially arranged plots imposes another limitation, for advantage cannot be taken of adjacency-correlations. With long strips, as in Beaven's "half-drill-strip" form of trial, adjoining strips are correlated for yield. So, too, are corresponding parts, *e.g.* single sheaves from such strips. But corresponding foot-length samples on adjoining strips, for instance the strips used in the experiment here described, show no significant correlation. This fact, demonstrated by study of the data, is to be expected. Number of plants per foot fluctuates greatly and the fluctuation is reflected in yield per foot. Early tillering, however, is not correlated with population density. Nevertheless, the data show that even for early tillering corresponding samples on adjoining strips are uncorrelated. The explanation of this probably lies in heterogeneity of soil and other factors. It may be surmised that field soils are marked by irregularity in the form of "patches"—it may be patches of five yards or more across. Correlations for yield between adjacent narrow strips or small plots reflect the existence of such patches. But within a patch are irregularities of a smaller order—"streaks" as it were. Clods, wire-worm attack, and hoof marks, are possible examples. Now small samples, as for instance foot-lengths of drill row, may often coincide

with such small irregularities which, because their influences are integrated, are not reflected in yield and other attributes of plots. It is apparent therefore that small samples have special statistical significance. Moreover they offer an interesting means of studying soil heterogeneity.

Correlation in connection with small samples (one-foot lengths of row) has been closely studied in earlier investigations (Engledow (2); Doughty and Engledow (3)). In a number of cases two samples, adjoining foot-lengths of row, were taken at every sampling point. There proved to be no significant correlation between members of these pairs for number of plants per foot, number of tillers or ears per plant, and yield per foot. By indirect means, however, the occurrence of a very low positive correlation was suggested. Even a low correlation would naturally affect certain estimates of probability. An example may be taken from the data of the 4th count in the present experiment. On the four combined untreated strips the following values were obtained in respect of number of side tillers per plant:

(i) Calculated by the "per-foot" method, *i.e.* mean of per-foot averages:

$$M = 0{\cdot}602 \;:\; \sigma = 0{\cdot}404 \;:\; n = 100 \text{ (foot lengths).}$$

(ii) Calculated by the "per-plant" method, *i.e.* total tillers on 100 samples divided by total plants:

$$M = 0{\cdot}593 \;:\; \sigma = 0{\cdot}798 \;:\; n = 1356 \text{ plants.}$$

Number of plants fluctuated sharply from one foot-length to another. Its arithmetic mean was 13·56, its harmonic mean 10·40. Using this latter value for number of plants per sample, the standard deviation of average number of tillers per plant (on foot-lengths) should be $0{\cdot}798/\sqrt{10{\cdot}4}$, or roughly 0·25. As shown above the actual value was 0·404. Probably a number of circumstances contribute to this difference. Inability to allow for size of tiller involves, as already explained, a rigid "grouping" of observations. This applies to both per-foot and per-plant methods; but the per-foot averages naturally give a wider and more symmetrical distribution for any population than the per-plant values. As a low correlation possibly subsists between the populations of adjoining foot-lengths it is probable that the plants belonging to any one foot-length show some degree of correlation among themselves. If this be so the value of $\sigma = 0{\cdot}798$ from the per-plant method is not a valid index to the standard error of the mean of a sample, for the per-plant observations from 100 samples are not independent. Any

correlation of the kind here indicated must presumably arise from soil factors. It cannot be an effect of fluctuating spatial interval as among foot-lengths, for in early development population-density and tillering are uncorrelated. There is thus difficulty in deciding upon the best test of significance. On the whole it has appeared desirable to employ the per-foot method, although the per-plant method always gives a narrower margin of allowance for errors due to sampling.

In the data for the 4th count, given above, mean number of tillers per plant is not widely different for the two methods of calculation. At later counts, when population-density and tillering show significant negative correlation, the divergence between the two means is far wider.

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VARIATION IN THE COMPOSITION OF THE MILK OF AN ABNORMAL COW.

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(With Two Text-figures.)

CONSIDERABLE attention has been directed in recent years to researches into the variation in the composition of milk of herds of cows, and the data available renders it possible to calculate by statistical methods the range of variation in composition of the milk of herds of various sizes. Nevertheless the authors have reason to believe that cows giving milk of abnormal composition are not uncommon in many herds, and it is therefore important to collect as much data as possible from the analysis of the milk of such cows, with a view to establishing the cause of abnormality in composition. It is even more desirable in such cases to know whether the mechanism of secretion of the milk has been so profoundly modified as to interfere with the correlations usually obtained between the individual constituents of milk.

In the course of an investigation into the composition of the milk of a herd of cows in Leicestershire during 1923–26 (1) the milk of several of the cows was sampled individually and analysed. The milk from one cow—Starlight—showed great abnormality in composition, sufficiently so to warrant the collection of data over a long period. It is the purpose of this paper to present and discuss the analytical records of the milk and observational data concerning this cow, during the period May 1924–July 1927.

HISTORY OF THE COW.

Starlight—a non-pedigree shorthorn—was purchased by the owner of the Leicestershire herd, mentioned above, from a farm in Derbyshire in 1921, her previous history being unknown. She dropped her third calf in December 1921, and her subsequent milk yield record and dates of calving are as follows:

Date of calving	Period in milk	Yield during lactation
23. xii. 21	278 days	9472 gallons
26. iii. 23	249 ,,	7539 ,,
7. iv. 24	316 ,,	8695 ,,
19. viii. 25	400 ,,	7529 ,,
2. iii. 27	123 ,,	5172 ,,

In June 1925 Starlight was bought by the Midland Agricultural College, and a veterinary examination at that time failed to reveal any

incipient signs of disease, or any factor which would account for the abnormally poor milk which she had produced. In all respects she appeared to be a healthy cow, and, moreover, one of a good dairy type.

From May 1924 until September 1926 samples of the morning's and evening's milk were taken on one day per week, and analysed. During the last lactation—March to July 1927—samples were taken on three consecutive days per week. The composition of the milk throughout was consistently abnormal, the percentages of fat being very variable, whilst the solids not fat content fell, in the majority of cases, well below 8·5 per cent. During the winter of 1925–26, the possible effect on the milk of feeding mineralised against non-mineralised cake was investigated, and during the last lactation rations containing varying amounts of protein were fed, with a view to ascertaining whether the composition of the milk could be influenced by radical changes in the balance of the ration.

On four occasions during 1924, the milk from all four quarters was tested with brom cresol purple paper, with the following results:

Date	Reaction
13. v. 24	All quarters normal
8. vii. 24	All quarters slightly alkaline
1. ix. 24	All quarters very slightly alkaline
24. xi. 24	Two quarters slightly alkaline, the other quarters appreciably alkaline

As previously noted, veterinary examination in June 1925, *i.e.* subsequent to the above tests, failed to reveal any signs of disease.

During March 1927 the milkers reported that one quarter was becoming hard, and the flow of milk restricted. An examination by a veterinary surgeon confirmed the milkers' report. A sample of milk was submitted to microscopical examination for tubercle bacilli but none was found to be present. Two tuberculin tests—Nov. 1926 and May 1927—were made, and the results showed a positive reaction. On two occasions during the spring of 1927 milk from each teat was weighed and analysed, with the following results:

Quarter	Yield in lb.	Fat %	Solids not fat %	Weight in lb. of Fat	Solids not fat	Total solids
April 4						
Right fore	6¼	3·15	7·98	0·197	0·499	0·696
Left fore	5	2·85	8·02	0·142	0·401	0·543
Right hind	2	3·20	7·35	0·064	0·147	0·211
Left hind	3	2·80	7·54	0·084	0·226	0·310
May 9						
Right fore	9¼	6·90	7·54	0·638	0·697	1·335
Left fore	6¾	7·80	7·77	0·526	0·524	1·050
Right hind	1	4·00	6·65	0·040	0·066	0·106
Left hind	5½	5·50	7·09	0·302	0·390	0·692

It is obvious from these data that the two hind quarters were seriously affected, the right hind being particularly bad.

Towards the end of June the cow showed signs of serious illness, and at the beginning of July she was slaughtered. Post-mortem examination revealed extensive tubercular lesions in the right lung, whilst the left organ was slightly affected only. The disease was very evident in the right hind quarter, but the remaining three were only slightly diseased. Her general condition was poor, but not excessively so.

ANALYTICAL DATA.

Two hundred and forty-nine samples of milk from this cow were analysed during three lactations. Maximum, minimum and mean figures are given in the following table:

Table I. *Percentage composition of the milk.*

	Fat	Solids not fat	Protein	Ash	Lactose (diff. figure)	Soluble ash	In-soluble ash	Ratio ins./sol. ash	P_2O_5	CaO	Ratio P_2O_5/CaO
1924 lactation. 33 samples. 2nd month to end of lactation											
Max.	3·80	8·80	3·48	0·88	4·55	Not determined			0·215	0·178	1·52
Min.	1·25	5·30	2·54	0·67	1·69				0·121	0·092	0·78
Av.*	2·48	7·19	2·71	0·77	3·71				0·169	0·134	1·26
1925–26 lactation. 114 samples. Complete lactation											
Max.	7·60	8·48	3·39	0·82	4·75	0·529	0·462	1·430	0·237	0·192	1·65
Min.	1·45	6·43	2·62	0·68	2·81	0·300	0·279	0·338	0·108	0·100	0·99
Mean†	3·409 ±0·064	7·703 ±0·028	2·965 ±0·009	0·768 ±0·002	3·975 ±0·028	0·382 ±0·003	0·379 ±0·002	1·017 ±0·013	0·180 ±0·001	0·145 ±0·001	1·202 ±0·009
1927 lactation. 102 samples. 1st month to date of slaughtering											
Max.	6·70	8·70	Not determined								
Min.	1·25	7·42									
Mean†	3·52 ±0·067	8·07 ±0·018									

* Arithmetical † Statistical.

DISCUSSION.

Fat. In the 1924 lactation the fat percentage decreased as the period advanced. In the subsequent lactation the percentage was variable, the lowest values occurring during December to June. In the last lactation, however, when the cow was obviously ill, extraordinary fluctuation occurred, ranging from 1·75 per cent. to 6·70 per cent. in the case of the morning's milk, and from 1·25 per cent. to 5·70 per cent. with respect to the evening's milk. Occasions when the percentage of fat in the morning's milk exceeded that of the preceding evening's milk were quite numerous, the percentage of such cases being 19 in the 1924 lactation, 39 in the 1925–26 lactation and 64 in the last lactation.

Solids not fat. Of the 249 samples analysed, 5 only contained more than 8·5 per cent. solids not fat. Twenty-four samples (10 per cent. of total) were below 7 per cent. in this constituent. In all three lactations the solids not fat content fell rapidly towards the end of the period, a variation which is decidedly abnormal.

Protein. The percentage of this constituent remained fairly steady during each of the two lactation periods in which it was determined. The mean figure (see Table I) is much lower than that obtained by Tocher (2) for individual cow's milk, or by the authors (3) in the case of mixed milk.

Lactose. Although the percentages given under this heading are difference figures only, they are sufficiently comparative to warrant inclusion. The mean percentage of this constituent is very low compared with that obtained for normal milk by other workers.

Ash. In the case of the 1924 lactation, there was a steady rise from July to the end of the period (November), but in the succeeding lactation a similar rise was not shown. The mean ash percentage is only slightly higher than that recorded by the authors for mixed milk, and is the one constituent which gave normal results.

Insoluble and soluble ash. In both lactations in which these constituents were determined, the insoluble ash fell steadily during the period, whilst the soluble ash varied in the reverse direction. Compared with the mean results recorded by the authors for mixed milk, the milk from this cow gave a much higher soluble ash percentage and a correspondingly lower insoluble ash percentage.

Ratio insoluble/soluble ash. This ratio declined as lactation advanced. The mean is markedly lower than that recorded in the case of mixed milk (3).

Phosphoric acid and lime. There was a tendency for both these constituents to fall as the period of lactation advanced, and this fall was intensified during the last three months of the period. The mean percentages of these constituents are much lower than one would expect to find in the case of mixed milk.

Ratio phosphoric acid/lime. This ratio fluctuated considerably during the first four months of the lactation, became steady later, and finally, in the last three months, fell to a value approximating to 1. On two occasions the percentage of CaO actually exceeded that of P_2O_5, a marked abnormality. The mean figure for the ratio is rather lower than that given by normal mixed milk.

In the following table is given the extent of variation of the means

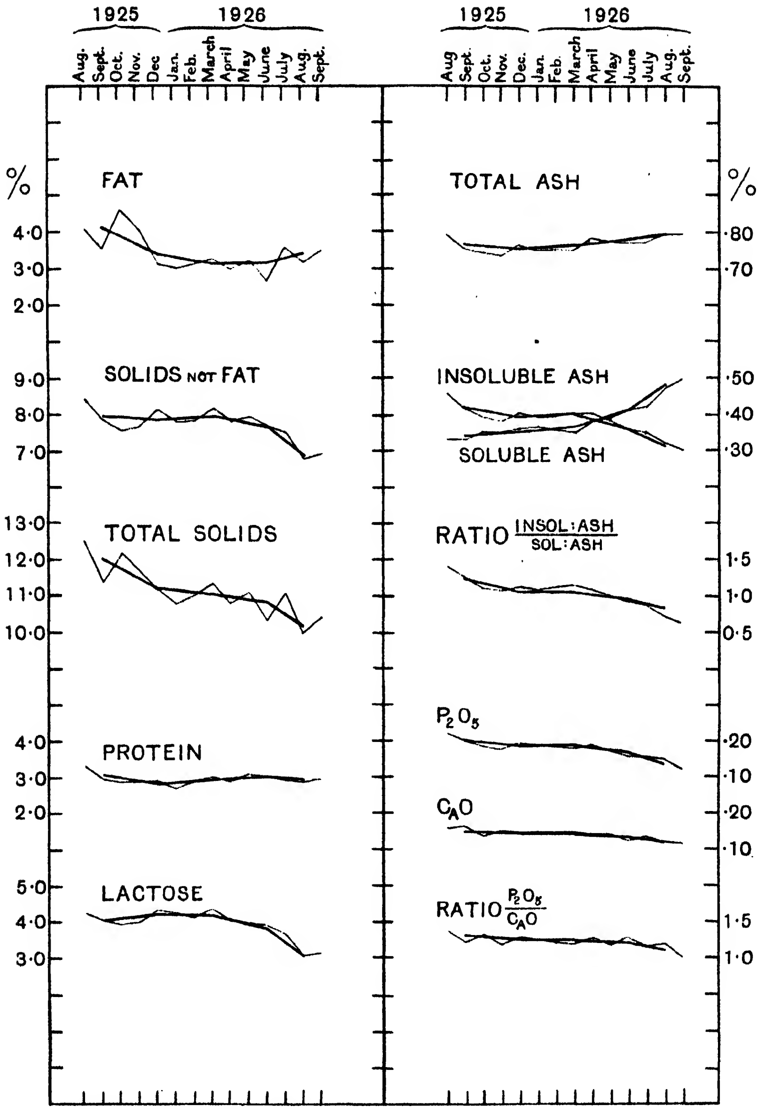

Chart 1. Variation in the composition of Starlight's milk during complete lactation.

———— Monthly averages   ━━━━ Tri-monthly averages

of the various constituents (obtained from the 1925–26 lactation data) from the means of normal mixed milk obtained by the authors in a previous investigation (3).

	Relative values of the means obtained from 114 samples of Starlight's milk (1925–26 lactation), taking the means for normal mixed milk = 100 respectively
Fat	92
Solids not fat	88
Protein	91
Lactose (diff. fig.)	84
Ash	101
Soluble ash	78
Insoluble ash	144
Ratio insol./sol. ash	55
Phosphoric acid	77
Lime	79
Ratio P_2O_5/CaO	94

The greatest variations from the normals are shown by the ash constituents and lactose, whilst the total ash content exhibits the least variation. Despite the abnormal percentages of the soluble constituents in the milk from this cow, the relationship between the lactose and

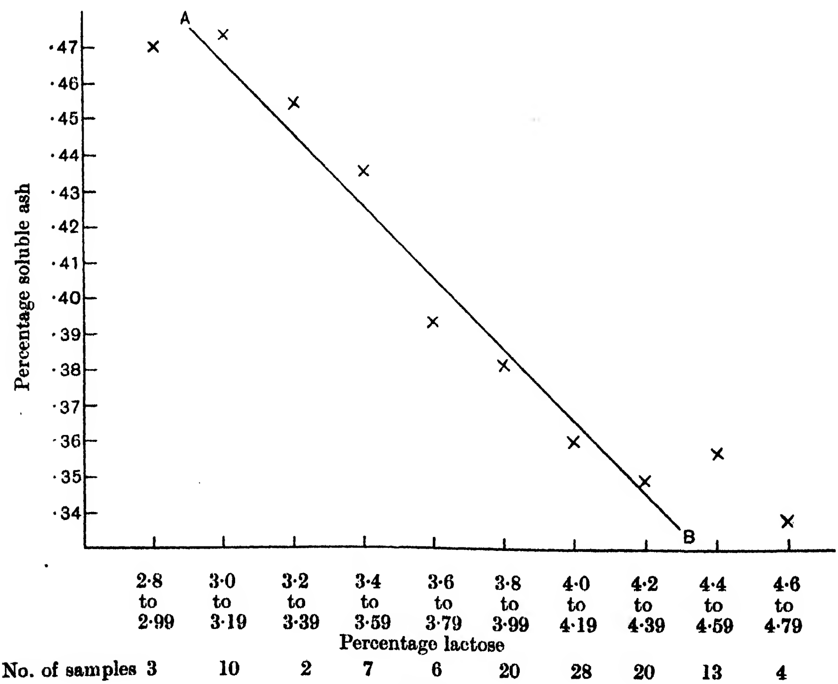

Chart 2.

soluble mineral constituents—a function of the osmotic equilibrium obtaining during milk secretion—is normal. The correlation between the lactose and soluble ash percentages is shown in Chart 2. The straight line *AB* has been drawn in accordance with Porcher's calculation (5) that a variation of 1 per cent. lactose in milk is accompanied by an inverse variation of 0·1 per cent. chloride. This marked negative correlation between these two constituents has been referred to previously by the authors (3).

From investigations carried out in Switzerland by Koestler (4) it appears that low lactose and high chloride figures are to be met with in the milk of cows suffering from disorders of secretion. From the data recorded in the case of Starlight, however, it will be observed that she was producing abnormal milk long before any signs of disease were apparent in an ordinary veterinary examination. In view of this remarkable case, one is inclined to suggest, tentatively, that abnormality in composition of milk may be a prior indication of some disease affecting the organs involved in the secretion of milk. In the case in question it does not appear that the abnormality in composition of the milk was hereditary, since Starlight's third calf—a heifer which came into milk in 1924—produced milk well up to average composition during a period of four months' observation. The results given in the following table will establish the fact that the milk from this heifer was normal in every respect.

Average composition of the milk of Starlight's heifer
(20 samples taken bi-weekly)

Fat	3·60	Insoluble ash	0·498
Solids not fat	8·89	Ratio insol./sol. ash	1·80
Protein	3·35	Phosphoric acid	0·247
Lactose (diff. fig.)	4·77	Lime	0·190
Ash	0·77	Ratio P_2O_5/CaO	1·31
Soluble ash	0·276		

Influence of feeding mineralised and non-mineralised cakes.

During the winter period of the 1925–26 lactation, supplies of special mineralised and non-mineralised cake (made by Messrs Silcock and Sons for the Rowett Institute) were obtained and fed to Starlight at the rate of 10 lb. per day, the remainder of her ration consisting of ryegrass hay. The differences shown by the average figures for each five weeks period are very small and of no significance. In the case of this cow the feeding of a non-mineralised ration for a period of five weeks, followed by an equal period during which a mineralised ration was fed, had no apparent effect on the low percentages of the various constituents of the milk.

Influence of feeding varying percentages of protein in the ration.

In the last lactation (1927) commencing in March, an attempt was made to alter the composition of the milk by varying the percentage of digestible protein in the ration fed to the cow. Digestible protein up to 0·93 lb. per day was fed, two periods of high protein feeding being separated by an interval during which the animal received pasture grass only.

Apart from the normal lactation fall, and the temporary improvement in quality due to the stimulating action of the grass, the only significant effect noted during this period of experimental feeding was an increase in the percentage of solids not fat from 8·1 to 8·3 in the ninth week, at the commencement of which the ration was changed from pasture grass to a high protein feed.

Summary.

This paper is a record of the composition of the milk of an abnormal cow, during a period extending over three lactations. Although this cow was found eventually to be suffering from tuberculosis of the udder and lungs, no signs of the disease were apparent during the first two lactations recorded.

The milk produced by this cow was abnormal during the whole period under review. Fat percentages were very variable, but the solids not fat content was consistently low, only 2 per cent. of the total number of samples analysed exceeding 8·5 per cent. in this constituent. Protein and lactose percentages were much below the averages for normal milk, but in the case of total ash the mean figures were normal. Of the ash constituents, the soluble portion was very high and the insoluble portion correspondingly low, the former presumably indicating a high chloride content. The percentages of phosphoric acid and lime were considerably below the mean figures for normal milk.

Lactose and soluble ash percentages show a marked negative correlation, and moreover support the contention of Porcher and others that a definite lactose-chlorine ratio exists in milk.

It is suggested that an abnormally low solids not fat content (*i.e.* low protein and lactose) and abnormal percentages of the individual ash constituents may be a sign of incipient disease affecting the organs involved in the secretion of milk.

Feeding with a "non-mineralised" cake for a short period, followed by a similar period in which a "mineralised" cake was fed, had no apparent effect on the composition of the milk produced.

Another period of experimental feeding, in which varying percentages of protein were included in the ration, followed by all grass feeding, was carried out. A temporary improvement in the quality of the milk due to sudden changes in the ration were observed, otherwise the composition of the milk was unaffected.

The authors wish to acknowledge the assistance of Miss D. G. Griffiths, B.Sc., A.I.C., who was responsible for a portion of the analytical work embodied in this paper.

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SOME OBSERVATIONS ON THE NITROGENOUS MANURING OF GRASSLAND.

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(With One Text-figure.)

In this paper an account is given of investigations undertaken during the years 1927 and 1928 on the nitrogenous manuring of grassland under a system of rotational grazing, now becoming widely known as "The New System of Grassland Management." The opportunity for making these arose when, in 1926, it was decided to devote to the "New System" an area of 45 acres of pasture previously manured only with phosphates and a little potash. The primary object of the change was to collect data over a series of years to throw light on the economic aspect of the matter, but it was decided also to collect as much information of a chemical and botanical character as the limited facilities of the Institute permitted. This paper deals mainly with the chemical and botanical data, leaving the economic side for fuller treatment at a later date.

Description of the grassland and arrangement of the investigation.

Prior to the Herts County Council purchasing the estate the grassland, on which the present investigations were made, served as the Park to the residence. It has probably been under grass for at least a century although the existence of ridges and furrows shows that earlier still it was arable land. When taken over by the Agricultural Institute in 1921 the grass was in a very bad state. Preliminary trials with various phosphates soon showed appreciable improvement and in succeeding years, by careful grazing with sheep and cattle, thorough harrowing, and the application of phosphates, the grass was rapidly improved and brought to a high standard.

Soil. The greater part of the 45 acres is on "Boulder Clay" but the eastern portion is on "Glacial Sand and Gravel." In order to have some idea of the condition of the soil at the commencement of the treatment

samples of the surface soil (to 9 in.) and of the sub-soil (9 to 18 in.) were taken from each of the plots. (These samples consisted of a number of cores taken at intervals along the diagonals of the plots.) Some measurements on these were kindly undertaken by the chemical department at Rothamsted. The area proved far more uniform than was expected; none of the plots had any reserve of chalk but all were neutral or slightly alkaline in reaction. The highest and lowest values for pH and replaceable lime are shown in Table I: all other figures were intermediate.

Table I. *Plots showing highest and lowest values for* pH *and replaceable lime.*

Plot		pH	Replaceable lime
4	Surface—to 9 in.	7·0	0·28
	Sub-soil—9 to 18 in.	7·0	0·29
2	Surface	7·0	0·21
	Sub-soil	7·0	0·22
3	Surface	7·3	0·23
	Sub-soil	7·4	0·23

During the winter 1926–27 the 45 acres were divided, by fences, into eight plots, seven of five acres to be managed according to the "New System" and one of ten acres (No. 8) to be treated in exactly the same way except that no nitrogen was to be applied. Water was provided for each plot. After the experience of the first season Plot 8 was subdivided into two plots, each of five acres, and Plot 3 became another no-nitrogen plot. Accordingly, "Plot 8" in 1927 refers to an area of ten acres which in 1928 became Plots 8 and 9. Of these two, Plot 8 continued to be a no-nitrogen plot while Plot 9 became another nitrogen plot.

The aim of the "New System" is to apply rapidly acting nitrogenous manures in order to get quickly a five or six inch growth of grass which is then eaten down closely in the course of eight to twelve days. The stock are moved to another plot, the one grazed is harrowed, another dressing of nitrogenous manure is applied and after a period of a few weeks it is ready again for heavy stocking. For the sake of efficiency the grazing is done by two groups of animals, the more important (at "Oaklands" the Dairy Herd) being put on first for a period of three to six days; they are then removed to the next plot, the completion of the grazing being carried out by "followers" consisting of dry cows, store cattle, horses, etc. Table II, giving actual dates of grazing and manurial dressings during 1927, will make the sequence of operations quite clear. The similar record for 1928 is omitted.

Table II. *Dates of nitrogen dressings and grazings*, 1927.

	Plot 1	Plot 2	Plot 3	Plot 4
1st N dressing	3. ii.	7 and 8. ii.	14. ii.	22. ii.
Preliminary grazing	22. iii. –25. iii.	26. iii. –29. iii.	30. iii. – 2. iv.	3. iv. – 6. iv.
1st complete grazing	22. iv. – 1. v.	27. iv. – 6. v.	2. v. –11. v.	7. v. –16. v.
2nd N dressing	3. v. (S.A.)	7. v. (S.A.)	12. v. (N.C.)	17. v. (S.A.)
2nd complete grazing	7. vi. –19. vi.	15. vi. –28. vi.	20. vi. – 2. vii.	29. vi. – 7. vii.
3rd N dressing	20. vi. (S.A.)	29. vi. (N.C.)	4. vii. (N.C.)	9. vii. (S.A.)
3rd complete grazing	26. vii. –14. viii.	2. viii.–21. viii.	9. viii.– 1. ix.	16. viii.– 7. ix.
4th N dressing	17. viii. (S.A.)	23. viii. (N.C.)	Not given	12. ix. (S.A.)
4th grazing	21. ix. –10. x.	27. ix. –16. x.	6. x. –21. x.	12. x. –23. x.

	Plot 5	Plot 6	Plot 7	Plot 8
1st N dressing	1. iii.	8. iii.	14. iii.	—
Preliminary grazing	7. iv. –10. iv.	11. iv. –14. iv.	15. iv. –18. iv.	19. iv. –22. iv.
1st complete grazing	12. v. –23. v.	17. v. –30. v.	24. v. – 6. vi.	31. v. –14. vi.
2nd N dressing	25. v. (N.C.)	31. v. (N.C.)	7. vi. (N.C.)	—
2nd complete grazing	3. vii. –19. vii.	8. vii. –22. vii.	15. vii. –25. vii.	21. vii. – 4. viii.
3rd N dressing	19. vii. (S.A.)	25. vii. (N.C.)	27. vii. (N.C.)	—
3rd complete grazing	26. viii.–13. ix.	5. ix. –20. ix.	8. ix. –24. ix.	14. ix. – 5. x.
4th N dressing	23. ix. (N.C.)	23. ix. (N.C.)	30. ix. (N.C.)	—
4th grazing	15. x. –25. x.	18. x. –28. x.	29. x. – 2. xi.	2. xi. – 4. xi.

The preliminary grazing was very light, carried out with sheep only.
There was ample growth for the 4th grazing on Plots 1, 2, 3 but very little on the others.
The first nitrogen dressing was 1 cwt. sulphate of ammonia on all seven plots.
Subsequent dressings were 1 cwt. sulphate of ammonia (S.A.) or its equivalent in nitro-chalk (N.C.).

Rainfall. Measurements of rainfall and other meteorological observations are made daily at the Institute but have not proceeded for a sufficient number of years to give reliable averages. The average rainfall for the County of Hertford is 26·29 in. and it may be assumed that the "Oaklands" figure is not far from this.

During the two seasons of the experiment the rainfall has been

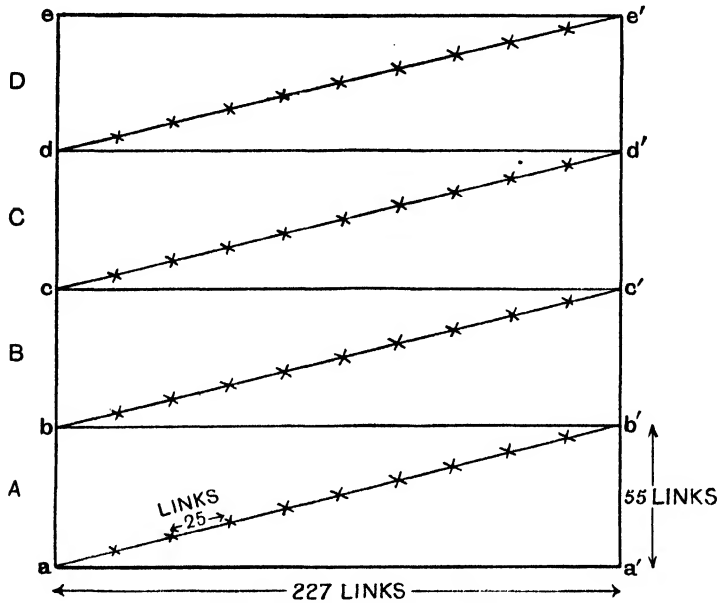
Oct. 1926–Sept. 1927, 812·2 mm. (= 32·0 in.)
Oct. 1927–Sept. 1928, 623·8 mm. (= 24·6 in.)

The difference between the two seasons is brought out more clearly by dividing the months into three periods.

Period	Rainfall year 1926–27	Rainfall year 1927–28
Oct. to March	384·3 mm.	400·6 mm.
April and May	71·6	53·9
June to Sept.	356·3	169·3
	812·2	623·8

It will be seen that the earlier part of 1928 resembled that of 1927 in the occurrence of a dry period in April-May but thereafter the two years were very different. Whereas, after May, 1927 was exceptionally wet, in 1928 the sub-normal rainfall persisted through the summer and autumn months.

Technique. For the economic data a daily record is compiled of all animals grazing the plots and from this record, by means of a table of equivalents, the total number of cow-day equivalents of grazing provided by each plot is obtained. At the outset of the trial, however, it was felt that some other method of estimating the production of herbage should be attempted and after considering a number of suggestions the following method was evolved and adopted.



Plan 1. Method of sampling plots.

On each of four plots (in 1927 Plots 2, 6, 7 receiving nitrogen and Plot 8 no nitrogen; in 1928 Plots 2 and 7 receiving nitrogen, and Plots 3 and 8 no nitrogen) there was selected a representative half-acre area free from interfering influences such as gates, water troughs, and trees, and approximately square in shape. These areas were divided into four strips, *A*, *B*, *C*, *D*, as shown in Plan I, the corners being defined by cutting out turves. On the day prior to the cows first entering a fresh plot a number of square yards of herbage were cut at equidistant intervals along the four diagonals, *ab′* (or *a′b*), *bc′* (or *b′c*), *cd′* (or *c′d*), *de′* (or *e′d*), of the strips *A*, *B*, *C*, *D*. These squares were marked out by inserting metal skewers at the corners and half-way along the sides of a wooden framework which enclosed exactly one square yard. The framework was

then removed and the grass within the skewers cut as closely as possible with ordinary garden shears, raked up with a small iron rake, put into numbered waterproof bags, and taken to the laboratory. Here, as each was weighed, it was carefully sub-sampled, a composite sample being obtained for each of the strips *A*, *B*, *C*, *D*. As soon as possible, duplicate portions were weighed out from each of the four composite samples for dry matter determinations and other portions from two of the strips, usually *A* and *D*, for subdivision into "grasses," "clovers," "other species" (or "weeds") and "waste" (dried grass, earth, dung, etc.). By this means it was possible to estimate the total weight of herbage available for the animals on first entering the plots, the total weight of dry matter and its chemical composition, the contribution of the grasses, clovers, weeds and waste to these totals, the chemical composition of the grasses, etc., and, if desired, the proportions by weight of the actual species present.

Test of method. At first, six square-yard cuts were made along each of the four diagonals of the selected area (*i.e.* 24 in all). On calculating the probable error of the average of the 24 weights of herbage per square yard it was found that the values of the P.E. of the mean of 24 sq. yd. samples were, when expressed as a percentage of the mean, 2·9 for Plot 6, 3·5 for Plot 8, 3·3 for Plot 2. These figures were reasonably low.

A second test applied was to sample the area on Plot 2 on two successive days in a period of dry weather when there was no dew and growth had practically ceased. On the first day samples were cut along the diagonals *ab′*, *bc′*, *cd′*, *de′*: on the second day along diagonals *a′b*, *b′c*, *c′d*, *d′e* (Plan I). The results were:

	Av. yield per sq. yd.	P.E. of mean
First day	437 gm.	3·3 % of mean
Second day	468	3·3 ,,

Although these results were fairly satisfactory it was decided to take 9 samples from each strip in future, *i.e.* 36 in all, in order to reduce the P.E. to 2·5 per cent. Succeeding values were actually slightly lower than this until the autumn, when the grass became more patchy and the P.E. rose to 3·1 per cent. During the long spell of dry weather in 1928 the grass became more and more uneven until the P.E. reached the high figure of 10 per cent. in October after having been 3·0 per cent. in June.

Size of sample. To decide if smaller areas than 1 sq. yd. would be satisfactory, twelve square-yards and twelve half-square-yards

($4\frac{1}{2}$ sq. ft.) were cut on the same strip of grass at the same time. The results were:

	Average	P.E. of mean
12 sq. yd. samples	880 gm.	4·8 % of mean
12 half sq. yd. samples	468	8·1 ,,

Hence, although the averages happen to agree quite well, the fluctuation in the case of the smaller samples is large and would have involved taking about 100 samples to obtain the same accuracy as 36 samples of 1 sq. yd. each. The latter method has, therefore, since been used.

SUMMARY OF ECONOMIC DATA 1927 AND 1928.

This summary is confined to a record of the amount of grazing provided by the plots. As already mentioned, the information is obtained by keeping a careful diary throughout the grazing season. For conversion of the different classes of stock into terms of cows the following equivalents were used:

1 cow = 1 horse.
= 7 half-bred sheep.
= 10 cross-bred lambs.
= 2 yearling cattle.
= 1 two-year-old cattle.

The total cow-days of grazing provided by each plot in 1927 and 1928 are shown in Table III.

Table III. *Summary of grazing in* 1927 *and* 1928.

Season 1927 Grazing period 23. iii.–5. xi.		Season 1928 Grazing period 9. iii.–8. x.	
Nitrogen plots	Total cow-days per acre	Nitrogen plots	Total cow-days per acre
No. 1	250	No. 1	183
,, 2	240	,, 2	197
,, 3	240	,, 4	179
,, 4	209	,, 5	184
,, 5	231	,, 6	218
,, 6	227	,, 7	189
,, 7	221	,, 9	193
Average	231	Average	192
No-Nitrogen plots	Total cow-days per acre	No-Nitrogen plots	Total cow-days per acre
No. 8	148	No. 3	150
		,, 8	168

In 1927 the order of grazing the plots was 1, 2, 3, 4, 5, 6, 7, 8, Plots 1 to 7 being 5 acres each and Plot 8 (no N) 10 acres. In 1928 the order of grazing was 1, 2, 3, 7, 8, 9, 4, 5, 6, each of the plots being 5 acres and

Plots 3 and 8 controls. In 1927, therefore, the strictest comparison for the effect of the nitrogen dressings (a total of 4 cwt. sulphate of ammonia or its equivalent per acre) is between Plots 7 and 8. Plot 7 yielded 221 cow-day equivalents per acre, Plot 8 148, figures in the ratio 149 to 100.

In 1928, the nitrogen Plots 2 and 7 may be compared with the no-nitrogen Plot 3, and nitrogen Plots 7 and 9 with the no-nitrogen Plot 8: but, taking soil variations and other factors into consideration, the strictest comparisons are between Plot 2 and Plot 3 and between Plot 7 and Plot 8. The cow-days for these respectively were 197 and 150 : 189 and 168. In the former pair the ratio is 131 : 100, in the latter pair 112 : 100. Unfortunately, owing to the small number of no-nitrogen plots little weight can be attached to these figures and the most that can be deduced is that possibly the increase in stock-carrying capacity produced by the nitrogen in 1927 was about 50 per cent., in 1928 about 20 per cent. (In 1928 the total nitrogen used was equivalent to 3 cwt. sulphate of ammonia per acre.)

A number of objections can be urged against the use of cow-day equivalents as a measure of the value of the herbage produced. The most obvious is the scale of equivalents which is necessarily arbitrary, but this is not very important when the same stock are being used for all the plots. A more important one is that this method lumps together all grazing days, productive or non-productive. Thus, a dry cow, acting as a "follower" and getting a maintenance ration from the grass, counts the same as a cow producing several gallons of milk. But the most serious objection is, that the decision when to move the animals from one plot to another is made by the manager, who, for convenience in management or other reasons, may keep the animals on a particular plot when they are getting insufficient nutriment from it. Because they are on the plot, grazing days are credited to it, though the after-effects—a fall in milk or in live weight—may not be felt until the animals have been removed to another plot. In practice such occasions arise fairly frequently, but, since the number of rounds of grazing in the season is only four, they are not sufficiently frequent to be assumed to "cancel out" by being evenly distributed over the plots. A further difficulty arises when supplementary foods are used either on the plots themselves or in the cowshed. For these reasons it is felt that the method of using grazing days as a measure of the production of herbage cannot be regarded as satisfactory and that a more accurate one needs to be devised.

BOTANICAL RESULTS, 1927 AND 1928.

In the hope of detecting any changes in the character of the pasture brought about by the "New System" a considerable number of botanical observations have been made. They have consisted mainly in analyses carried out by the "percentage area covered" method, the "specific frequency" method and a new method based on the technique already described. In the percentage area method a 100 sq. in. frame (20 × 5 in.), divided into inch squares by fine wires, is placed upon the piece of turf selected as representative of the area to be analysed and, working inch by inch, the area covered by each species is estimated. The process is repeated for as many turves as possible and the figures obtained are averaged. Bare space also is estimated. In the specific frequency method a small frame, 6 × 6 in., is placed on the grass at definite intervals along selected diagonals of the area to be analysed and a note made of the species occurring within it. (The number of times a species occurs within the frame is not noted, but only if it does occur.) The number of occurrences for a particular species are added up and calculated as a percentage of the total possible occurrences. If the frame is placed down n times the highest possible total for any species is n.

The value of these two methods depends largely on the objects in view. The percentage area method is of value for comparing two pieces of sward in the same season if at comparable stages of growth, or to compare the same piece of sward in successive seasons. It is, however, very tedious, and must be used when the grasses are short and depends for its accuracy on the judgment of the analyst in selecting the turves. The second method could be employed to determine whether particular species are disappearing or coming in, but beyond this does not seem capable of giving much useful information—indeed, if employed for comparing two pastures, might be entirely misleading. The great drawback of both methods is that they do not give a reliable measure of the actual production of herbage during the course of a season. The percentage area method fails because it must be employed when the grass is short so that the importance of dwarf and broad-leafed species is likely to be overestimated. The specific frequency method, while it may be used when the grass is long, fails because the number of occurrences of a species within the frame is not recorded and also because it does not take into account at all the very different characters of the species (amount of leafiness, etc.).

For these and other reasons it was decided, during the latter part of

Table IV. *Production of herbage and its botanical composition.* 1927.

Date of sampling (1)	Plot No. (2)	Days since last grazing (3)	Rain since last grazing mm. (4)	Av. yield wet weight cwt. per acre (5)	Dry matter % (6)	Av. yield dry matter cwt. per acre (7)	Botanical composition of herbage by weight as % of total wet matter: Grass (8)	Clovers (9)	Weeds (10)	Waste (11)	Prob. error of mean yield as % of mean (12)
1st recorded grazing round											
21. v.	7	33	17·9	86·0	21·55	18·25	72·0	1·9	10·7	15·4	3·6*
30. v.	8	38	32·7	41·8	27·6	11·5	74·2	4·5	17·0	4·3	3·5*
13. vi.	2	38	39·9	44·7	30·45	13·6	87·3	2·6	3·2	6·9	3·0*
2nd recorded grazing round											
15. vii.	7	38	123·0	145·5	14·18	20·6	87·1	1·8	5·0	6·1	2·3
21. vii.	8	37	122·3	111·2	15·76	17·6	75·6	8·7	2·9	12·8	2·3
2. viii.	2	35	71·9	162·0	14·74	23·9	89·0	2·85	3·1	5·05	2·2
3rd recorded grazing round											
8. ix.	7	44	98·9	183·0	15·29	28·0	89·8	1·0	1·4	6·8	2·3
15. ix.	8	41	147·0	131·5	14·54	19·1	82·5	6·9	5·2	5·4	2·2
27. ix.	2	36	125·5	103·0	14·96	15·4	88·3	0·8	3·6	7·3	3·1

Plots 7 and 2 received four dressings of nitrogen each equivalent to 1 cwt. sulphate of ammonia during 1927. Plot 8 received no N.

* In the 1st round only 24 sq. yd. samples were cut: subsequently 36 squares were cut.

Table V. *Production of herbage and its botanical composition.* 1928.

Date of sampling (1)	Plot No. (2)	Days since last grazing (3)	Rain since last grazing mm. (4)	Av. yield wet weight cwt. per acre (5)	Dry matter % (6)	Yield dry matter cwt. per acre (7)	Botanical composition of herbage by weight as % of total dry matter: Grass (8)	Clovers (9)	Weeds (10)	Waste (11)	Total waste per acre cwt. (12)	"Edible" dry matter cwt. per acre (13)	Prob. error of mean yield as % of mean (14)
							1st grazing round						
30. iv.	2	43	60·1	37·0	20·94	7·82	83·0	0·8	6·2	10·0	0·78	7·04	4·5
7. v.	3	46	61·0	41·3	21·36	8·80	76·0	3·1	12·1	8·8	0·77	8·03	4·1
14. v.	7	28	8·5	37·3	21·00	7·85	78·7	0·9	7·5	12·8	1·01	6·84	3·4
18. v.	8	23	19·0	40·0	19·02	7·62	77·8	3·8	10·9	7·5	0·57	7·05	3·1
							2nd grazing round						
25. vi.	2	35	37·8	55·7	27·51	15·27	83·5	2·9	3·5	10·1	1·54	13·73	3·0
29. vi.	3	37	43·2	57·6	25·70	14·82	80·1	8·1	5·5	6·3	0·93	13·89	3·7
6. vii.	7	40	56·1	70·6	23·09	16·34	85·8	1·2	3·4	9·6	1·57	14·77	2·9
11. vii.	8	42	56·1	24·7	28·90	7·05	83·0	5·65	3·0	8·35	0·60	6·45	6·2
							3rd grazing round						
2. viii.	2	26	42·2	9·3	28·80	2·67	43·6	1·1	0·8	54·5	1·45	1·22	5·4
6. viii.	3	26	61·6	18·0	25·75	4·63	38·3	4·7	6·8	50·2	2·32	2·31	2·9
9. viii.	7	23	64·4	31·5	24·30	7·64	57·7	0·85	2·05	39·4	3·01	4·63	2·9
14. viii.	8	25	65·2	22·25	27·12	6·05	59·5	4·2	1·9	34·4	2·08	3·97	4·2
							4th grazing round						
20. ix.	2	34	42·1	14·5	28·42	4·12	67·25	0·45	0·9	31·4	1·29	2·83	6·9
24. ix.	3	35	42·1	11·15	33·96	3·80	53·1	2·4	4·6	39·9	1·52	2·28	6·1
27. ix.	7	32	31·7	13·3	31·49	4·20	37·3	0·8	1·4	60·5	2·56	1·64	8·9
2. x.	8	34	33·3	6·4	36·07	2·33	37·1	2·4	1·6	58·9	1·37	0·96	10·2

Column 13 = Column 7—Column 12. Column 12 is calculated from Columns 7 and 11.

Plots 2 and 7 receive N. Plots 3 and 8 receive no N.

the 1927 season, to utilise a part of the "grasses" obtained in the chemical laboratory, when the two strip composite samples had been divided up into "grasses," "clovers," "weeds," and "waste." After reserving what was required of the fresh "grasses" for chemical analysis, the remainder was subdivided until a suitable quantity was obtained, and this then carefully separated into species. These were dried at 100° C. and the dry weights obtained. The separation was usually done in duplicate. By this method a fairly accurate estimate was obtained of the specific contributions by weight to the total quantity of herbage produced.

General botanical analysis. Tables IV and V, Cols. 8, 9, 10, 11. In the early part of 1927, while methods were still being developed, the separated grasses, clovers, weeds and waste were not dried but were weighed in the wet state only; later in the year, facilities for drying these samples were arranged so that both wet and dry weights were obtained. In the tables the botanical composition for 1927 is calculated throughout on the wet weight of herbage: for 1928 the percentages are on the dry weight.

The most striking feature of the botanical analysis is the small weight contributed by the clovers. In previous years, *i.e.* before the use of any nitrogenous manures, botanical analyses had been carried out by the percentage area method after manuring with phosphates. The results showed the percentage of clover as varying from 14, on badly grazed, to 33 on well grazed, areas. It was, therefore, very surprising to get such low figures as 4·5 per cent. or even 8·7 per cent. on the control, Plot 8, in 1927; these results suggested that the percentage area method of botanical analysis may greatly overestimate the amount of leguminous species in the herbage.

Table VI. *Weight of clover (cwt. per acre before drying).*

	1927			1928			
Round	1	2	3	1	2	3	4
Plot 7 (N)	1·63	2·62	1·83	0·35	0·99	0·61	0·16
Plot 8 (no N)	1·88	9·65	9·09	1·60	1·62	1·22	0·23
Ratio 8 : 7	1·15	3·68	4·97	4·57	1·63	2·0	1·43

As regards the effect of nitrogen on the botanical composition, it will be seen that in every one of seven rounds recorded in Tables IV and V the percentage of clovers in the nitrogen plots is well below that in the controls: on the average the latter have about four times as much clover as the former. It is extremely unlikely that this is a chance result and it is reasonable to conclude that, in spite of the close grazing to which the plots have been subjected, the clovers have been suppressed

by the nitrogen. This conclusion is confirmed when actual weights per acre of clover are considered instead of percentages. The figures for two plots, Plot 7 receiving nitrogen and Plot 8 a control, are set out in Table VI.

Table VII. *Relative percentages by weight of grass species on four plots*, 1928.

Round No. / Species	Plot 2 (Nitrogen) 1	2	3	4	Plot 3 (Control) 1	2	3	4
Lolium perenne	31·05	32·8	Not determined	70·85	44·2	35·8	Not determined	69·7
Poa pratensis and *trivialis* chiefly *trivialis*	17·0	17·0		3·70	17·6	14·2		3·35
Agrostis spp.	17·6	14·7		13·95	14·2	14·2		15·0
Holcus lanatus	6·1	8·9		5·0	6·35	5·6		3·65
Anthoxanthum odoratum	5·5	5·1		0·75	3·1	3·0		1·35
Festuca ovina	5·7	3·1		1·8	3·95	6·0		3·65
Cynosurus cristatus	3·8	10·8		—	3·8	5·6		0·15
Dactylis glomerata	—	—		—	3·7	0·6		0·35
Alopecurus pratensis	7·95	3·8		—	0·45	5·4		—
Phleum pratense	—	0·15		—	—	0·6		—
Miscellaneous	1·8	0·15		—	1·7	1·6		—
Odd bits	3·5	3·50		3·95	0·95	6·4		2·65
*Luzula campestris**	—	—		—	—	1·0		0·15

Round No. / Species	Plot 7 (Nitrogen) 1	2	3	4	Plot 8 (Control) 1	2	3	4
Lolium perenne	19·4	14·9	21·5	44·8	30·15	36·7	38·7	51·25
Poa pratensis and *trivialis* chiefly *trivialis*	35·3	24·45	17·35	7·15	21·7	17·6	11·85	6·2
Agrostis spp.	16·2	20·6	27·3	27·2	16·15	18·4	17·85	21·25
Holcus lanatus	10·8	14·9	9·5	13·9	10·5	9·75	6·2	9·9
Anthoxanthum odoratum	3·8	2·8	2·8	0·5	7·3	3·5	3·85	2·5
Festuca ovina	6·5	6·0	3·35	3·4	4·85	3·7	2·15	4·4
Cynosurus cristatus	0·4	2·9	0·35	—	1·95	2·9	2·55	0·25
Dactylis glomerata	1·3	1·0	1·55	2·05	0·2	2·3	1·45	0·70
Alopecurus pratensis	1·0	3·9	—	—	2·75	1·05	—	—
Phleum pratense	0·1	1·2	—	—	—	—	0·15	1·05
Miscellaneous	1·2	0·35	2·8	—	1·15	0·20	—	1·8
Odd bits	4·0	6·8	13·5	0·85	3·3	3·50	15·25	0·7
*Luzula campestris**	—	0·2	—	0·15	—	0·4	—	—

* Overlooked in preliminary separation.

In 1927, an abnormally wet season, the nitrogen Plot 7 was able to produce much more herbage than Plot 8; in 1928, with an abnormally dry summer, the nitrogen dressings produced little, if any, effect on the weight of herbage during the third and fourth rounds. So far as the weight of clover is concerned, Plots 7 and 8 start off very similarly in 1927 (1·63 cwt. and 1·88 cwt. respectively). In the third round, however, Plot 8 produces 9·09 cwt. of clover against 1·83 on Plot 7. In 1928 the

two plots start from the relative position reached in 1927, as shown by the ratio 8 : 7. By the fourth round, however, the amounts of clover on the two plots are not greatly different, suggesting that treated and untreated plots become very similar in clover content when, owing to absence of rainfall, the nitrogen is not visibly acting. Apparently it is a case of suppression (probably indirect) and not of elimination. As Plot 3 was not a control in 1927 the same analysis cannot be applied to the pair of Plots 2 and 3.

The species of grasses and their contribution to the herbage. During 1928, with the exception of the third round on Plots 2 and 3, on each occasion of sampling, a portion of the separated grasses was divided up into species which were then dried and weighed. The results, calculated as percentages of the total dry weight of grasses, are set out in Table VII. In nearly every case the figures are averages of two samples, in some cases of four samples. Accordingly, while it is not possible to attach importance to small differences or changes in the percentages, yet it is felt that Table VII does give a substantially accurate picture of the specific contributions to the herbage during the season. The chief points are:

1. Perennial Rye Grass, throughout the season and particularly after the long period of dry weather, has held an important position: in the fourth round it has constituted between 45 and 71 per cent. of the total weight of grass.

2. The two Meadow Grasses (chiefly Rough Stalked), at the beginning of the season comparable with Rye Grass in amounts, have fallen to an insignificant portion during the dry weather.

3. The other two important species are Bent and Yorkshire Fog. The former has maintained or increased its relative importance during the season: the latter has kept very steady.

4. With the exception of Plot 2, Cut 2, Crested Dog's Tail has been of little importance.

5. Sheep's Fescue has contributed about 5 per cent. and has remained steady at this level.

6. None of the other species make any important contribution to the herbage.

7. There is nothing at present to suggest specific reactions of the different grasses to the application of nitrogen, *i.e.* the above statements apply to both N and no-N plots.

It is hoped to continue this analysis during subsequent years, in which case important deductions should be possible.

Chemical results, 1927 and 1928.

So far, it has only been possible to complete fully the dry matter and nitrogen determinations for the very large number of samples obtained in the two years. The dry matter has been determined by drying for 48 hours or longer at 100° C.; for the total nitrogen the ordinary Kjeldahl method has been applied to the dry matter after grinding and redrying. In 1928 a certain number of check nitrogen determinations were made on the wet herbage; the figures obtained agreed substantially with those for the dried samples. A considerable number of determinations of the ash and silica have been made but the results are not included as they have not yet been completed. It is also hoped to perform a number of nitrogen estimations on the dried samples of grass species which have been separated out.

The method of sampling has already been described but may be recapitulated here (see Plan I). Except for the early part of 1927 when only 24 sq. yd. were used, for each sampling of the selected areas 36 separate sq. yd. were cut, bagged separately in waterproof bags, taken to the laboratory and weighed. A composite sample was then made up from the first nine bags (Strip *A*), another from the bags 10–18 (Strip *B*), another from bags 19–27 (Strip *C*), and another from bags 28–36 (Strip *D*). From each of these strip composites, duplicate samples were taken for dry matter, and the dry matter after grinding was used for nitrogen determinations. Accordingly, dry matter figures for the plots are the average of eight determinations (two for each strip), while nitrogen figures for the plots are the average of at least four determinations (one for each strip, duplicated where necessary). As already explained, the term "waste" is applied to the part of the herbage, obtained during the preliminary botanical separation, which consists of dried-up grass, earth, dung, etc.

Production of dry matter. The chief initial object in sampling the plots was, as already mentioned, to measure the production of herbage on the nitrogen and control plots as it was felt that "grazing days" was an unsatisfactory method. The figures obtained for three plots in 1927 and for four plots in 1928 are included in Tables IV and V, Column 7, and are extracted and summarised in Table VIII. In 1928, owing to the high percentage of "waste" during the dry weather, the weight per acre was calculated and a corrected figure for the "edible" dry matter is shown in Table V (Col. 13) and in Table VIII.

The most striking feature of Table VIII is the difference between

1927 and 1928. Thus the nitrogen Plot 2 produced in three rounds in 1927 52·9 cwt. of dry matter per acre: in 1928, in four rounds, exactly the same area produced 29·9 cwt. The no-nitrogen Plot 8 in 1927 produced 48·2 cwt. in three rounds, in 1928 only 23·05 cwt. in four rounds. It is clear that rainfall has been the chief factor controlling the growth of the herbage.

Table VIII. *Summary of production of dry matter (cwt. per acre).*

1927

Plot ...	2 Nitrogen cwt.	7 Nitrogen cwt.	8 No Nitrogen cwt.
1st grazing round	13·6	18·25	11·5
2nd " "	23·9	20·6	17·6
3rd " "	15·4	28·0	19·1
Total	*52·9*	*66·85*	*48·2*

Nitrogen applied to 2 and 7: four doses, each equivalent to 1 cwt. sulphate of ammonia per acre.

1928

Plot	2 Nitrogen		3 No Nitrogen		7 Nitrogen		8 No Nitrogen	
Round	Gross cwt.	Corrected for waste cwt.	Gross cwt.	Corrected for waste cwt.	Gross cwt.	Corrected for waste cwt.	Gross cwt.	Corrected for waste cwt.
1	7·82	7·04	8·80	8·03	7·85	6·84	7·62	7·05
2	15·27	13·73	14·82	13·89	16·34	14·77	7·05	6·45
3	2·67	1·22	4·63	2·31	7·64	4·63	6·05	3·97
4	4·12	2·83	3·80	2·28	4·20	1·64	2·33	0·96
Total	29·88	24·82	32·05	26·51	36·03	27·88	23·05	18·43

Plots 2 and 7 nitrogen plots (three doses each equivalent to 1 cwt. sulphate of ammonia). Plots 3 and 8 no nitrogen.

Considering next the effect of the nitrogen dressings in 1927, both the nitrogen plots, 2 and 7, produce more herbage than the control Plot 8 but they differ very much from one another. A considerable interval elapsed between sampling 2 and 7 but only a short one between 7 and 8, which for this and other reasons are much more comparable. Plot 7 produced in the three rounds 39 per cent. more dry matter than Plot 8, a figure agreeing roughly with the estimate obtained by grazing days. Little importance, however, can be attached to results from a single pair of plots. In 1928 there are two pairs of plots, 2 to be compared with 3 and 7 with 8. Between 2 and 3 the difference is slight and is in favour of the no-nitrogen plot: in the case of 7 and 8 there is a considerable difference in favour of the nitrogen plot. Again, therefore, little can be deduced from the figures and it must be admitted that, so far, the methods employed have failed to provide a reliable measure of

the relative production of herbage on the nitrogen and no-nitrogen plots. Fortunately, however, other data of interest have been derived from the chemical investigations.

Table IX *a*. *Protein percentages and yields of protein per acre*, 1927.

	Nitrogen				No Nitrogen	
	Plot 2 Protein		Plot 7 Protein		Plot 8 Protein	
Round	In dry matter %	Cwt. per acre	In dry matter %	Cwt. per acre	In dry matter %	Cwt. per acre
1	15·0	2·10	15·5	2·83	13·2	1·52
2	18·35	4·38	19·0	3·92	15·1	2·66
3	20·7	3·19	18·1	5·07	17·8	3·40
Average	18·0	3·22	17·5	3·94	15·4	2·53
Total	—	9·67	—	11·82	—	7·58

Table IX *b*. *Protein percentages*, 1928.

	Nitrogen				No Nitrogen			
	Plot 2		Plot 7		Plot 3		Plot 8	
Round	In total dry matter %	Corrected for waste %	In total dry matter %	Corrected for waste %	In total dry matter %	Corrected for waste %	In total dry matter %	Corrected for waste %
1	22·90	24·9	21·00	22·8	18·30	19·4	17·20	18·0
2	15·05	15·5	13·59	14·0	14·53	14·9	14·66	14·0
3	15·87	23·0	18·77	24·0	15·44	20·0	16·05	20·5
4	18·74	20·5	14·38	18·5	15·76	19·3	13·24	18·75
Average	18·14	21·0	16·94	19·8	16·01	18·4	15·29	17·8

Protein, cwts. per acre, 1928.

	Plot 2		Plot 7		Plot 3		Plot 8	
Round	In herbage as cut	Corrected for waste	In herbage as cut	Corrected for waste	In herbage as cut	Corrected for waste	In herbage as cut	Corrected for waste
1	1·79	1·75	1·65	1·56	1·61	1·56	1·31	1·27
2	2·30	2·13	2·22	2·07	2·15	2·07	1·04	0·99
3	0·42	0·28	0·96	0·81	0·71	0·46	0·96	0·81
4	0·77	0·58	0·61	0·32	0·60	0·44	0·30	0·18
Total	5·28	4·74	5·44	4·76	5·07	4·53	3·61	3·25
Average	1·32	1·19	1·36	1·19	1·27	1·13	0·90	0·81

During the dry spring spell of 1927 the highest dry matter content was recorded on Plot 2, 30·45 per cent. on June 13. Thereafter no figure above 16 per cent. is recorded for the Plots 2, 7, 8. The seasonal range of variation is from 14·18 per cent. (Plot 7, July 15), up to the figure 30·45 per cent. In 1928 only one figure below 20 per cent. is recorded, viz. Plot 8, May 18. (Some rain had fallen on the 15th and 16th, accounting

for this result being below the plots previously sampled.) The highest for the season is the last obtained, 36·07 per cent. on Plot 8, October 2, when the yield of dry matter was only 2·3 cwt. per acre. On the same plot in September 1927 the percentage of dry matter was 14·54, with a yield per acre of 19·1 cwt. Nothing could more clearly bring out the difference between the two seasons.

Protein percentages and yields of protein per acre. Tables IX *a* and IX *b*. These figures throughout refer to crude protein, *i.e.* nitrogen × 6·25. In 1927 for any particular round the percentage of protein in the dry matter is invariably higher on the nitrogen plots, and on the average is 2·3 above the no-nitrogen plots. The total production of protein for the three rounds averages 10·75 cwt. for the nitrogen plots, and is 7·68 on the control, a difference big enough to be significant. In the case of the 1928 figures, owing to the high percentage of waste in the samples taken during the dry weather, it has been deemed advisable to calculate the columns "corrected for waste." This allows for the dry matter and crude protein contributed by the "waste" portion of the herbage. In this year, comparing the percentage of protein on Plot 2 with 3, and on 7 with 8, the figure for the nitrogen plots is, on the average, again 2·3 above the controls. As regards total production Plot 7 has given nearly 50 per cent. more than Plot 8, but 2 and 3 are about equal. Comparing the two years the average percentage for the same plot is only slightly different (*e.g.* Plot 2, 18·0 in 1927 and 18·14 in 1928), but the total production differs greatly. Plot 7 in 1927 averaged 3·94 cwt. of crude protein per acre per round but in 1928 only 1·36: the corresponding figures for Plot 2 are 3·22 and 1·32, for Plot 8, 2·53 and 0·90. Thus in 1927 there is between 2½ and 3 times as much protein produced as in 1928.

Table X. *Selected results showing variations in yield, dry matter, and protein of neighbouring strips.*

Plot and round	Strip	Av. yield of strip wet weight per sq. yd. gm.	Dry matter in wet herbage %	Crude protein in dry matter %
Plot 2, round 2	*B*	1651	13·77	19·7
(1927)	*C*	1760	15·47	17·8
Plot 7, round 1	*B*	958	20·42	16·5
(1927)	*C*	909	23·22	14·8
Plot 8, round 2	*A*	1180	16·53	14·6
(1927)	*B*	1174	14·68	17·2
Plot 8, round 2	*A*	206	28·9	15·3
(1928)	*B*	175	31·5	13·2

Relationship between percentage of dry matter and percentage of crude protein. Examination of the detailed figures for the strip composite samples in 1927 showed that the production on adjacent strips *A*, *B*, *C*, *D* of a particular half-acre area, as measured by the nine-square-yard samples, varied considerably. The biggest difference was as much as 25 per cent. of the lower sample. Since these variations are several times the P.E. they cannot be attributed solely to errors in sampling. Further, the strip samples showed significant differences in dry matter and protein content. A few of the most striking of these samples are included in Table X.

Table XI. *Percentage dry matter and protein in the separated constituents of the herbage,* 1928.

			Dry matter in wet constituents				Crude protein in dried constituents			
Plot	Round	Date	Grass %	Clovers %	Weeds %	Waste %	Grass %	Clovers %	Weeds %	Waste %
2	1	30. iv.	21·5	19·7	15·8	71·0	23·2	27·8	22·7	4·5
3	1	7. v.	22·7	19·6	14·7	50·0	18·75	27·7	22·6	6·5
7	1	14. v.	21·6	23·1	15·2	60·7	22·1	28·7	24·4	9·23
8	1	18. v.	19·4	19·5	15·6	35·6	17·5	25·3	17·9	6·25
Av.	1		21·3	20·5	15·3	54·3				
2	2	25. vi.	23·4	23·1	18·8	58·3	15·5	23·9	16·2	10·75
3	2	29. vi.	29·6	22·1	21·5	66·5	14·2	24·65	15·5	9·1
7	2	6. vii.	25·7	22·7	16·8	28·7	13·7	22·0	15·3	9·65
8	2	11. vii.	30·8	30·0	23·4	79·5	16·25	22·9	13·7	9·0
Av.	2		27·4	24·5	20·1	58·25				
2	3	2. viii.	28·0	25·3	20·0	47·6	22·97	27·75	31·40	9·93
3	3	6. viii.	26·5	24·3	16·2	56·5	20·85	23·88	15·94	10·57
7	3	9. viii.	25·15	13·6	13·2	55·95	23·3	15·76	27·0	10·51
8	3	14. viii.	27·2	25·0	20·8	55·5	19·28	25·5	23·25	7·29
Av.	3		26·7	22·05	16·05	53·9				
2	4	20. ix.	29·0	30·25	23·7	59·7	20·37	28·05	26·30	14·59
3	4	24. ix.	33·2	31·9	15·4	69·5	18·59	26·86	22·62	10·29
7	4	27. ix.	32·9	28·1	18·7	52·3	18·31	25·95	23·60	11·44
8	4	2. x.	32·8	28·3	15·3	61·2	15·76	23·37	26·10	9·13
Av.	4		32·0	29·6	18·3	60·7				

Percentage dry matter

	Grass	Clovers	Weeds	Waste
Average of all	26·85	24·15	17·45	56·8

Percentage crude protein in dried constituents

	Grass	Clovers	Weeds
Average of samples from 2 and 7 (N plots)	19·93	26·31*	23·36
Average of samples from 3 and 8 (no N)	17·65	25·02	19·70

* Omitting Plot 7, round 3.

Although the number of examples is insufficient to establish definitely any relationship they suggest that, if on the occasion of a particular

sampling the herbage of one strip has a distinctly higher percentage of dry matter than that on an adjacent strip, its dry matter will be distinctly less rich in nitrogen, and *vice versa.* The results in both years emphasise the necessity for a large number of samples in sampling areas even though these areas be apparently uniform and no larger than ¼ or ½ acre.

Protein in the separated constituents. Table XI. During the third round in 1927 a start was made with reserving sufficient of the dried constituents—grasses, clovers, weeds and waste—for the purpose of determining the crude protein in these. The average figures obtained were: Grasses 18·55 per cent., clovers 24·1 per cent., weeds 18·5 per cent., waste 8·25 per cent. The differences were sufficiently great to justify continuing the work in 1928: the full results for that year are shown in Table XI.

With one exception, viz. Plot 3, Cut 3 (after which a leak in the distillation apparatus was detected but lack of material prevented a repetition), the clovers have very much more protein than the grasses and weeds. Averaging all the figures the results are: Grasses 18·79, clovers 25·67, weeds 21·53, figures in the ratio 100 : 137 : 115 respectively. It is clear that the clovers are much superior to the grasses as suppliers of protein both in the wet period of 1927 and throughout 1928. In the third round of 1927 the weeds and grasses were about equal, but in 1928 the weeds are, on the average, 15 per cent. better than the grasses, the difference being most marked in the driest period. As regards the influence of the manuring, it is evident from the summary in Table XI that the dressings of nitrogen have considerably increased the percentage of protein in the grasses and weeds, and to a slighter extent, that in the clovers.

Dry matter in the separated constituents. Table XI. Since the constituents were weighed before as well as after drying the percentages of dry matter in the constituents of the freshly cut herbage can be calculated. This was done for the third round in 1927 and throughout 1928. The 1928 figures are included in Table XI: they show that during 1928 the grasses have a higher percentage of dry matter than the clovers and these in turn more than the weeds, the ratios being

Grasses	Clovers	Weeds
100	90	65

During the third round of 1927, in a period of wet weather, the corresponding ratios were 100, 115, 72, *i.e.* the grasses had less dry matter than the clovers. Combining these results with those of the preceding

section it will be seen that clovers as grazed are between 30 and 40 per cent. better than grasses as suppliers of protein; weeds, owing to their higher moisture content are below grasses, though their dry matter may be equal to, or better than, that of the grasses.

Effect of age on composition. It was part of the scheme of work for 1928 to sample at least two plots at intervals between the cessation of one grazing period and the beginning of the next, in other words, between the dates of the samplings already recorded. It was hoped by this method to obtain a record of the rate of production of dry matter and protein and also to determine the protein content of grasses, clover and weeds at various ages. Actually, owing to the dry weather, it was only possible to do this once as, during the rest of the summer, the grass was too short for the method to be applied. Although little importance can be attached to one set of results they are recorded in Table XII along the line marked "interim." To make comparison easier figures for the prior and subsequent samplings are also given in the table.

Table XII. *Composition of two interim samples and comparison with prior and subsequent samples.*

Mixed herbage

Plot	Sample	Date of cutting	Days since last grazing	Rainfall since last grazing mm.	Yield wet cwt. per acre	Dry matter %	Dry matter cwt. per acre	Crude protein % in dry matter	Crude protein Cwt. per acre
2	1st rd.	30. iv.	43	60·1	37·0	20·94	7·82	22·9	1·79
	Interim	8. vi.	18	11·9	34·5	23·9	8·24	20·3	1·67
	2nd rd.	25. vi.	35	37·8	55·7	27·51	15·27	15·05	2·30
3	1st rd.	7. v.	46	61·0	41·3	21·36	8·80	18·3	1·61
	Interim	18. vi.	26	35·2	46·7	24·75	11·52	15·6	1·79
	2nd rd.	29. vi.	37	43·2	57·6	25·70	14·80	14·53	2·15

Constituents of herbage

Plot	Sample	Date of cutting	% of dry weight of herbage due to Grass	Clovers	Weeds	Waste	% crude protein in dry matter of Grass	Clovers	Weeds	Waste
2	1st rd.	30. iv.	83·0	0·8	6·2	10·0	23·2	27·8	22·7	4·5
	Interim	8. vi.	71·5	1·4	3·9	23·2	20·75	27·6	22·05	13·75
	2nd rd.	25. vi.	83·5	2·9	3·5	10·1	15·5	23·9	16·2	10·75
3	1st rd.	7. v.	76·0	3·1	12·1	8·8	18·75	27·7	22·6	6·5
	Interim	18. vi.	77·6	4·4	8·6	9·4	15·3	24·4	15·7	9·4
	2nd rd.	29. vi.	80·1	8·1	5·5	6·3	14·2	24·65	15·5	9·1

In the case of Plot 2 the interim sampling was done almost exactly half-way between the end of the first grazing period and the beginning of the second. The rainfall, however, was not evenly distributed, 11·9 mm. falling during the 18 days preceding the cutting of the interim sample as against a total of 37·8 mm. for the total period of 35 days preceding the

second round sample. Under these circumstances the results were that the amount of dry matter per acre at the end of 18 days was estimated at 8·24 cwt. containing 1·67 cwt. crude protein (20·3 per cent.); at the end of 35 days the estimate was 15·27 cwt. dry matter with 2·30 cwt. crude protein (15·05 per cent.), *i.e.* the amount of dry matter had approximately doubled but the protein had only increased by about one half. Between the 18th and 35th day the percentage of protein had fallen from 20·3 per cent. to 15·05 per cent.

In the case of Plot 3 the interim sampling could not be done until the 26th day after the end of the previous grazing. The weight of dry matter per acre was estimated at 11·52 cwt. and the protein content was already down to 15·6 per cent. Eleven days later the weight of dry matter was 14·80 cwt. and the percentage of protein had fallen to 14·53 per cent. In the period of eleven days only 8 mm. of rain had fallen but there was little change in the dry matter content of the fresh herbage. No definite conclusion can be drawn from these two interim samples but they suggest that by the end of the third week the crude protein in grass is usually not above 20 per cent. In the extensive series of investigations at Cambridge during the years 1925 and 1926 (1, 2) when grass was cut at weekly intervals the percentage of crude protein averaged, in both seasons, 24·74. In 1927 when grass was cut at fortnightly intervals (3) the average figure for protein was only slightly lower, viz. 23·48. Probably, therefore, by the end of the third week and certainly by the end of the fifth week the crude protein in the dry matter of grass will fall from the high figures of 23 to 25 per cent. obtained in the Cambridge experiments to the figure averaging between 17 and 20 per cent. obtained in the "Oaklands" investigations.

Composition of the tips of growing grass. A minor observation during the course of the first season throws some light on the reason for the high protein content obtained at Cambridge. Towards the end of the summer of 1927 it was observed that the cows, on being turned into a fresh plot, confined their grazing largely to biting off the tips of the herbage. In the course of a day or two the whole of the plot had been thoroughly "tipped" and then the cows proceeded to graze down what remained. There was also a suggestion in the milk yields that, after the "tipping" had been finished, there was a falling off in yield. Accordingly, with a view to getting some clue to the reason for this practice (which might be attributed to "succulence") some special samples were cut on Plot 2, on September 23, 1927, quite close to the area which was to be sampled a day or two later as part of the normal routine. Ten one-square-

yard areas were marked out, the tips cut off (about an inch or so long) and put into one bag, and the remainder of the herbage cut and put into another bag. The average weight of "tips" was 80 gm. per sq. yd.: the average weight of the "rest" 748·5 gm.

Samples were taken for dry matter, crude protein, etc., with the results shown in Table XIII.

Table XIII. *Analysis of "tips" and "rest" of herbage.*

Cut on Plot 2, Sept. 23, 1927.

	Proportion by weight wet	Dry matter %	Proportion by weight dry	Crude protein: In dry matter %	Crude protein: In wet matter %	Crude protein: Ratio in wet
"Tips"	9·7	17·72	11·4	27·2	4·8	170
"Rest"	90·3	14·41	88·6	19·5	2·8	100

Analysis of dry matter

	Crude protein	Crude oil	Soluble carbohydrates	Crude fibre	Ash	Containing silica
"Tips"	27·20	7·69	34·96	18·20	11·95	3·18
"Rest"	19·50	5·52	35·11	19·25	20·62	12·25

In the succulent "tips" the percentage of dry matter is 17·72, the "rest" is only 14·41. In the dry matter of the "tips" the crude protein is 27·2 per cent., in that of the "rest" 19·5 per cent. Calculating from these figures and the proportions of the "tips" and "rest" the crude protein in the complete herbage the figure obtained is 20·4, which agrees closely with the actual average for Plot 2 cut four days later (20·7).

In the material as cut, *i.e.* prior to drying, the percentage of crude protein is 4·8 per cent. in the "tips," 2·8 per cent. in the "rest," the ratio being 170 to 100. So far as crude oil (ether extract), soluble carbohydrates and crude fibre are concerned there are no important differences, but it is very significant that the dry matter of the "tips" has 11·92 per cent. ash, while that of the "rest" has 20·62 per cent. Accordingly, when consuming "tips" the cows, apart from any question of digestibility, needed to consume far less weight to get the necessary amount of dry matter and crude protein than when consuming the lower portions of the herbage; at the same time they avoided the large amount of ash in the "rest," which is about 60 per cent. silica.

SOME PRACTICAL CONSIDERATIONS.

From the botanical and chemical results described the following practical deductions can be made.

1. As the clovers in a pasture are considerably richer in protein than

the grasses then suppression under the intensive system tends to lower the quality of the herbage.

2. At an average age of five weeks the grass at "Oaklands" had a protein content of about 17 per cent. indicating that any supplement to this type of grass in the case of dairy cows should be of a "balanced" nature. Actually, however, cattle entering a plot with grass a month old have the choice, which apparently they exercise, for the first day or two, of securing a richer diet by "tipping" the grass. Thereafter the diet is less rich.

3. Cows grazing in plots containing from 15 to 20 cwt. dry matter per acre are obviously able to obtain their daily requirement of 25 to 35 lb. dry matter for maintenance and production purposes. The 1927 season provided abundantly in this respect. On the other hand, when the available dry matter per acre fell to 4 to 6 cwt., as it did in July 1928, supplementary feeding was required by the cows and the "followers" had obviously to work hard to maintain themselves. This suggests that the maximum area a beast can graze bare in a day is between $\frac{1}{15}$ and $\frac{1}{25}$ acre—which opens up a question of considerable practical importance in relation to the frequently repeated advice given to farmers to graze their pastures while the herbage is very young. If a young growth provides only 100 lb. dry matter per acre then an animal would have to graze no less than one-quarter of an acre to secure 25 lb. dry matter. There may, therefore, be a condition of close grazing, which, while securing the highest quality, yet reduces the quantity below a satisfactory level.

Summary.

An account is given of observations made during the first two years' treatment of grass according to the "New System of Grassland Management," *i.e.* periodic dressings of nitrogenous manure followed by rotational grazing.

The grazing provided by the nitrogen and no-nitrogen plots in the two years is given, measured in cow-day equivalents.

A method of sampling the plots with a view to determining the total weight of herbage produced and its chemical and botanical composition is described.

Botanical and chemical results obtained by this method are given and discussed.

The percentage of clover on the nitrogen plots is about one-quarter of that on the control plots.

The dry matter of the herbage on the nitrogen plots has, on the

average, contained 17·7 per cent. crude protein: that on the no-nitrogen plots 15·5 per cent. The average age of the grass when sampled was 35 days.

The percentage of dry matter in the components of the herbage grouped under grasses, clovers, weeds and waste, and the crude protein in these, are given. Weeds have much less dry matter than grasses and clovers; clovers are much richer in protein than grasses, weeds being intermediate. From this it is argued that the botanical composition may have important effects on the quality of the herbage produced.

An interesting habit of the cows grazing in the late summer of 1927 is described and analyses made as a consequence of this observation are given and discussed.

A point of practical importance in the grazing of stock is raised.

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THE AVAILABILITY OF THE POTASSIUM IN SOME SCOTTISH SOILS.

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CONTENTS.

The present paper contains the results of a comparison of some of the chemical methods commonly used in soil examination when applied to some Scottish soils. The methods used included Dyer's citric acid extraction, the standard English method of extraction with hot concentrated hydrochloric acid and the determination of the exchangeable potash. A few determinations of the total mineral potash have been made in order to obtain a comparison with the hydrochloric acid method. Some soils have also been examined by Neubauer's seedling method and the values obtained compared with the value of the exchangeable potash.

1. Experimental methods employed.

The total mineral potash was determined by the Lawrence-Smith method—0·5 gm. of the finely powdered soil was heated in a platinum crucible with a mixture of ammonium chloride and calcium carbonate and the soluble chlorides extracted from the residue with hot water and the potash determined in the resulting solution.

The HCl-soluble potash was determined by the standard English method—20 gm. of soil were treated with 70 c.c. constant boiling point hydrochloric acid for 48 hours on the water bath and the potash determined in an aliquot portion of the filtrate.

The citric soluble potash was determined by Dyer's original method—200 gm. soil were treated for seven days with 2000 c.c. 1 per cent. citric acid solution with frequent shaking and the potash determined in 500 c.c. of the filtrate. The exchangeable potash was determined by extracting 25 gm. of soil with a normal solution of ammonium chloride until 500 c.c. of filtrate were obtained.

The potassium was determined in all the extracts by the volumetric cobaltinitrite method of Morris (4). This method has been found to be very convenient and accurate for the small quantities of potash dealt with. In some preliminary experiments using the perchloric acid method the precipitate of potassium perchlorate was found invariably to contain traces of sulphate.

2. RESULTS OF ANALYSES.

Table I contains a list of the soils examined together with the depths to which they were sampled and brief geological and cultivation notes. In those cases where a complete profile was available the individual

Table I.

Location of soil sample and notes on geology and cultivation	Soil No.	Depth in inches	*p*H	HCl-soluble K_2O %	Citric soluble K_2O %	Exchangeable K_2O %
Boghall, Midlothian. Cultivated alluvial flat over basalt	548 *a*	0–6	6·37	0·213	·0025	·0051
	b	6–14	6·87	0·265	·0025	·0048
	c	14–26	6·78	0·331	·0028	·0055
	d	26–48	6·92	0·261	·0029	·0044
	e	48–60	6·95	0·334	·0014	·0036
Boghall, Midlothian. Cultivated alluvium over basalt	169 *a*	0–9	6·07	0·265	·0032	·0058
	b	9–18	6·19	0·250	·0020	·0048
Bolsham, Angus. Pine wood; thin boulder clay over andesite	843 *b*	4–11	4·63	0·290	·0031	·0065
	c	11–19	4·71	0·482	·0026	·0037
	d	19–39	5·84	0·616	·0032	·0060
Boghall, Midlothian. Cultivated glacial sand and gravel	509 *a*	0–9	5·92	0·251	·0038	·0066
	b	9–18	6·26	0·260	·0023	·0050
Craibstone, Aberdeenshire. Cultivated boulder clay over granite	399 *a*	0–9	5·59	0·490	·0035	·0088
Insch, Aberdeenshire. Cultivated thin boulder clay over norite	398 *a*	0–9	5·94	0·524	·0053	·0107
Humbie, East Lothian. Cultivated glacial sand and gravel over sandstone	672 *a*	0–9	6·78	0·191	·0062	·0116
	b	9–24	7·08	0·182	·0029	·0056
Old Fargie, Perthshire. Uncultivated grass; thin soil over andesite	593 *a*	0–6	5·62	0·310	·0074	·0189
	b	6–20	5·81	0·317	·0021	·0080
Polmont, Stirlingshire. Good grass, seldom ploughed; carse soil (alluvium)	589 *a*	0–8	5·26	0·742	·0102	·0195
	b	8–20	6·24	0·773	·0084	·0192
	c	20–48	7·68	0·711	·0146	·0246
	d	48–72	7·73	0·670	·0402	·0563
Glenfarg, Perthshire. Grass in open wood, uncultivated; thin soil over andesite	592 *a*	0–7	5·32	1·01	·0095	·0212
	b	10–24	5·36	0·374	·0013	·0049
Alva, Clackmannanshire. Cultivated but usually under grass; alluvial flat	591 *a*	0–8	5·90	0·136	·0124	·0217
	b	8–20	5·32	0·994	·0129	·0228
	c	20–40	5·53	1·12	·0144	·0254
Dunbar, East Lothian. Cultivated; thin boulder clay over limestone	587 *a*	0–8	6·70	0·342	·0145	·0230
	b	8–20	7·37	0·374	·0046	·0062
	c	20–36	7·07	0·673	·0047	·0059
Bridge of Earn, Perthshire. Grass at edge of stream; carse soil (alluvium)	590 *a*	0–7	5·79	0·369	·0285	·0490
	b	7–24	4·98	0·953	·0062	·0152
	c	24–48	4·86	1·09	·0066	·0136
	d	48–72	2·97	0·833	·0015	·0050

layers were analysed with the exception that a purely peaty or organic layer was ignored. The letters (*a*), (*b*) and (*c*), etc. merely refer to successive layers and not to the *A*, *B* and *C* horizons. The soils were all air-dried and the fraction passing the 3 mm. sieve used for the analyses.

The results of the determination of the HCl-soluble, citric acid soluble, and exchangeable potash are given in Table I, and the values obtained for the total mineral potash are given in Table II. The figures given are the means of at least duplicate determinations.

Table II.

Soil No.	589 *a*	590 *a*	591 *a*	592 *a*	593 *a*	548 *a*
Total mineral K_2O	2·51 %	3·00 %	2·13 %	1·87 %	1·51 %	1·22 %
HCl-soluble K_2O	0·742	0·369	0·136	1·01	0·310	0·213

3. Discussion of results.

All the soils examined show an acid reaction in the surface layer, but soils 672, 589 and 587 become alkaline in the lower layers. The basal material of soil 590 has the abnormally low *p*H of 2·97 and the soil was found to contain considerable quantities of water extractable aluminium sulphate. With two exceptions, soils 590 and 591, there is a decrease in acidity with increase in depth of sampling.

In the six soils examined the total mineral potash lies between 1·22 and 3·00 per cent., with an average value of 2·04 per cent. The three alluvial soils 589, 590 and 591 are much richer in potash than the three drift soils 592, 593 and 548. The relation to the HCl-soluble potash shows no kind of regularity, the percentage HCl-soluble to total varying from 6·6 to 54 per cent.

Robinson, Steinkoenig and Fry (7) have given the results of the total analyses of 34 American soils of widely different types and representative of considerable areas. The values of the potash range from 0·05 to 3·96 per cent., with a mean value of 1·00 per cent. Russell (8) has given the results of the analyses of 11 South of England soils in which the potash ranges from 0·30 to 1·44 per cent., with a mean value of 0·65 per cent. These figures would indicate that the Scottish soils examined possess comparatively large reserves of potash. This is in agreement with the evidence obtained from the mineralogical analyses of similar soils carried out by Hendrick and Newlands (3), who found that the Scottish soils contained larger quantities of unweathered mineral fragments than the South of England soils examined by them.

The quantities of potash soluble in hot strong hydrochloric acid vary from 0·14 to 1·21 per cent. of the air-dry soil and the average value for

all the soils is 0·500 per cent. and for the surface soils 0·395 per cent. In the profile samples there is generally an increase in soluble potash with increase in depth of soil, with one marked exception in soil No. 592. In soils 590 and 548 there is an indication of a layer of accumulation but the profile of soil 589 shows no accumulation and there is a slight decrease in solubility in the lower layers. The Carse soils are seen to be the richest in HCl-soluble potash. Soil 591 shows a remarkable increase in solubility with depth varying from the lowest to the highest value obtained in the series. This variation is not paralleled in the case of the citric soluble or exchangeable potash in this soil.

The quantities of potash extracted by the 1 per cent. citric acid solution show a much greater range of variation than do the quantities extracted by concentrated hydrochloric acid, varying from 0·0013 to 0·0402 per cent., with an average value of 0·0074 per cent. For the surface soils the values lie between 0·0025 and 0·0285 per cent., with a mean value of 0·0086 per cent. With but a few exceptions the quantities extracted and their ratio to the HCl-soluble potash decrease with increase in depth of soil. The most definite exception is soil No. 589, which contains nearly four times as much citric soluble K_2O in the basal material as in the surface soil. The proportion of the HCl-soluble potash extracted by the citric acid also varies considerably, the quantities extracted varying from 0·18 to 7·7 per cent. of the former. Discussing the relationship between the two methods of analysis, Russell (9) states that "the available potash shows no kind of regularity but varies between 5 and 50 per cent. of the quantity extracted by strong acids." In the Scottish soils examined this proportion is very much lower but there is also no regularity when compared with the HCl-soluble potash. The lower proportion may be due to the higher average value of the HCl-soluble potash combined with a lower value of the citric soluble or "available" potash. Russell gives the average value of the available potash in eight South of England soils as 0·017 per cent., which is practically twice the value for the Scottish soils.

The values obtained for the exchangeable potash vary from 0·0036 to 0·0563 per cent., with an average value for the samples from all the layers of 0·0135 per cent. The average value for the surface soils is slightly higher, being 0·0161 per cent. There is the same general decrease in quantity with increase in depth of soil as was observed in the case of the citric soluble potash. On comparing the potash present in the exchangeable form with that soluble in hydrochloric acid, the proportion is seen to vary considerably, ranging from 0·56 to 15·5 per cent. of the

latter. When a comparison is made between the exchangeable and citric soluble potash a fairly close relationship is seen to exist between the two quantities, the ratio of citric soluble to exchangeable varying from 26 to 79 per cent., with an average value of 52 per cent.

The product moment correlation coefficient between the two series, calculated in the usual manner, is found to be + 0·9786. In order to test the significance of this coefficient the method given by Fisher (1) was employed. For a coefficient of this value obtained from 34 samples $t = \frac{r}{1 - r^2} \cdot \sqrt{n - 2} = 26{\cdot}88$. For a probability $P = 0{\cdot}01$ that the two series are not correlated t should be about 2·75. Since P decreases with increase in t it is evident that there is an extremely small probability that the two series are not correlated.

This relationship is to be expected if we consider the citric acid extraction to be a case of partial base exchange. As the citric acid extract has a pH value of about 2·5 it cannot be expected to have much solvent action on the soil minerals, and if it is assumed that the action of the acid does not entirely destroy the power possessed by the soil of adsorbing bases it is evident that there must be a state of equilibrium in any such solution in contact with the soil. In the ordinary methods for determining the exchangeable bases this equilibrium is disturbed by making repeated extractions with fresh solvent until the quantity of potash in the extracts becomes vanishingly small.

Wiegner has shown that there is a dynamic equilibrium in the solution in contact with an adsorbing colloid, and this equilibrium is independent of concentration but dependent on the ionic ratio of ions in the solution. In the case of the citric acid extract the potash extracted would, therefore, depend on the cationic ratios in the extract, and on the nature of the undecomposed adsorbing complex. These two factors would naturally vary with each soil and would account for the variations observed between different soils. The fact that the quantity of potash extracted by the citric acid was always much less than the exchangeable content indicates that there could have been very little, if any, potash liberated by the citric acid apart from that previously existing in the exchangeable form.

The three soils Nos. 548, 169 and 509 from the Experimental Farm of the Edinburgh and East of Scotland College of Agriculture, Boghall, Midlothian, are very similar, being comparatively low in HCl-soluble and very low in citric soluble and exchangeable potash. The other glacial sand and gravel—No. 672—is also very low in HCl-soluble potash but

slightly richer in citric soluble and exchangeable. Soils 592 and 593 are very similar in origin, being thin soils over Andesite and probably residual. The citric soluble and the exchangeable figures are alike but there is quite a large difference in HCl-soluble potash, although the total mineral values for these soils are fairly similar. Soil No. 843, also over Andesite but with thin drift, is the only soil from a wood and the values of the citric soluble and exchangeable potash are much lower than the other Andesite samples, although the HCl-soluble content is similar to soil 593. The Aberdeenshire soils, Nos. 398 and 399, are derived from boulder clay over granite and norite respectively. The analytical results are similar, both soils containing moderately large quantities of HCl-soluble potash while the citric soluble and exchangeable values are comparatively low. The two Carse soils (alluvium) are among the richest of the soils examined, soil 590 containing twice as much exchangeable potash as any other examined. This soil has also the very high value of 3·00 per cent. total mineral potash. It may be noted that of the last eight soils six are either permanently or usually under grass vegetation, but it is impossible from the above data to draw definite conclusions regarding the effect of cultivation on the potash content of the soil.

The results obtained from 13 surface soils and one basal soil when examined by Neubauer's seedling method (5) are given in Table III, together with the corresponding values of the exchangeable potash. Six of the soils are from Boghall Farm, Midlothian, and of the others, four are from Aberdeenshire, two from Angus and one from Stirlingshire.

Table III.

Soil No.	Location	"Neubauer" K_2O %	Exchangeable K_2O %
1117	Boghall Farm, Midlothian	·0073	·0098
1118	,, ,,	·0053	·0081
1119	,, ,,	·0066	·0097
1120	,, ,,	·0056	·0082
976	,, ,,	·0081	·0098
1061	,, ,,	·0061	·0114
589 *a*	Near Polmont, Stirlingshire	·0129	·0195
589 *d* (basal)	,, ,,	·0424	·0563
1006	Silverhill, Aberdeenshire	·0070	·0085
1007	,, ,,	·0311	·0341
1008	,, ,,	·0078	·0102
1009	,, ,,	·0057	·0065
1059	Inverkeilor, Forfarshire	·0152	·0206
1060	Brechin, Forfarshire	·0258	·0317

Neubauer's average "limit-value" for the potash extracted by the seedlings is 24 mg. per 100 gm. soil or a content of 0·0240 per cent. On this basis the Boghall soils with about 5 to 8 mg. would appear to be

very deficient in available potash. Soils 1007 and 1060 are the only surface soils sufficiently supplied with potash and the basal soil 589 *d* at a depth of 4 to 6 feet is seen to be easily the richest of the soils examined.

When the Neubauer values are compared with the values of the exchangeable potash there is evidence of a rough correlation between the two quantities. The ratio of "Neubauer" to exchangeable varies from 53 to 91 per cent., with a mean value of about 74 per cent. This is somewhat higher than the mean value of 52 per cent. obtained from similar soils for the citric acid-exchangeable ratio, and emphasises the power of the young seedlings to absorb available potash from the soil. The correlation coefficient obtained between the values of "Neubauer" K_2O and exchangeable K_2O is $r = + 0{\cdot}9888$. From this we obtain a value of $t = 22{\cdot}94$. For a probability $P = 01$ the value of t should be about 3·05 and the extremely high value obtained indicates a correspondingly high probability that the two values are correlated.

Hasenbäumer and Bulks (2) describe some experiments in which quantities of K_2O in the form of potassium chloride were added to a soil and the mixture subsequently analysed by their 1 per cent. citric acid method and by the seedling method. For three soils the percentage extractions by the citric acid solution were 80, 61 and 67 per cent. (mean 69 per cent.) and by the seedling method 76, 78 and 91 per cent. (mean 82 per cent.). While the citric acid figures are not strictly comparable with the present data owing to the different technique, the figures for the seedling extraction are interesting and suggest that practically all the potash existing in a soil in the exchangeable form must be considered to be available to the plant, although the quantity available at any particular time is in all probability governed by several factors.

While the figures available at present are too few to allow of definite conclusions being drawn, it is probable that the quantity and nature of the replaceable bases largely determine the amount of cations which can be absorbed from the soil solution by the seedlings. Sven Odén (6) has published recently a very interesting paper containing the results of an exhaustive examination by means of electrodialyses of some Swedish and Dutch soils together with the results of experiments on the buffering capacity of soils in relation to their content of exchangeable bases. In order to demonstrate the importance of the replaceable ions in plant nutrition he extracted all the replaceable ions from a rich garden soil by means of electrodialyses; the dialysate was then added to pure

quartz sand and oats were grown on the sand and also on the extracted soil. The oats grown in the soil were unable to develop and it was shown that a considerable part of the mineral content of the seed was given off to the soil, which in this way became more saturated with bases. Odén concludes that "this demonstrates clearly that the growing plants obtain their nutritive salt ions only from the swarm of replaceable ions surrounding the soil particles and cannot dissolve or utilise any food from the soil minerals. The maximum amount of available salts in the soil is therefore identical with the replaceable ions."

If the relationship between the exchangeable potash and the Neubauer value is found to be general it follows that the exchangeable potash is a good indication of the manurial requirement of the soil for this nutrient since Neubauer's method has been found to be in fairly general agreement with the results of field experiments. It also has the great advantage of being independent of the conditions under which the analysis is carried out. Both Neubauer's method and the citric acid method have to be very carefully standardised before reproducible results can be obtained.

4. SUMMARY.

(1) The solubility of the potash in 34 soil samples from 13 soils typical of large areas in the East of Scotland has been determined by the methods of chemical analysis using (*a*) hot concentrated hydrochloric acid, and (*b*) 1 per cent. citric acid solution, and the values obtained compared with the quantities of potash existing in the exchangeable form. The total mineral potash has been determined for six samples and Neubauer's method of analysis has been applied to 10 samples and again compared with the exchangeable potash.

(2) The average value of the total mineral potash in the soils examined was 2·04 per cent., which indicates the presence of comparatively large reserves of potash in these soils. No relation was found to exist between the total potash and the quantities soluble in hot concentrated hydrochloric acid.

(3) The average value of the HCl-soluble potash was 0·50 per cent. and in the profile samples there was generally an increase in solubility with increase in depth of soil.

(4) The citric soluble potash had an average value of 0·0074 per cent. which represents only 1·48 per cent. of that soluble in strong hydrochloric acid. In the profile samples the solubility generally decreased with increase in depth of soil, with one marked exception in soil No. 587.

The citric soluble potash varied from 26 to 79 per cent. of the exchangeable potash with a correlation coefficient of + 0·9876, and it is suggested that the citric acid extraction is a case of partial base exchange.

(5) The figures obtained from the Neubauer seedling analysis indicate a deficiency of available potash in most of the samples examined. On comparison with the corresponding values of the exchangeable potash a correlation coefficient of + 0·9888 is obtained, which indicates that the soil's content of exchangeable potash determines the amount of K_2O which can be absorbed by the seedlings.

I wish to acknowledge my indebtedness to Dr W. G. Ogg of the Edinburgh and East of Scotland College of Agriculture for placing the samples of the above soils at my disposal and for his interest and assistance throughout the course of the work.

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A RAPID ELECTROMETRIC METHOD FOR DETERMINING THE CHLORIDE CONTENT OF SOILS.

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(With Two Text-figures.)

In the past the standard method for determining the chloride content of soils has been to titrate with silver nitrate using potassium chromate as indicator. In most cases, the soil must be filtered through a filter candle and where in favourable cases filtration can be dispensed with, it is necessary to wait until the soil has settled before aliquot portions can be pipetted off and titrated.

The method to be described has two great advantages over the older method. In the first place, it is much more rapid; the determinations are carried out on the soil suspension itself, and secondly, it is more accurate and less subject to personal error.

Theory of the method.

When a silver wire, coated with silver chloride, is immersed in a solution containing chloride ions, the potential difference set up at the metal-solution interface is given by the formula

$$E = E^0 - \frac{RT}{F} \ln \frac{1}{[Cl']},$$

where E^0 is a constant for this electrode having at 25° C. the value − 0·2245. (G. N. Lewis' conventions are adhered to throughout.)

At the theoretical end-point in the titration of a chloride solution with silver nitrate, the chloride ion concentration is $1{\cdot}0 \times 10^{-5}$ at 25° C.

The potential of the Ag-Ag Cl electrode in this solution is calculated from the above formula to be: − 0·521 volt at 25° C. Now if, in accordance with the principle laid down by Müller (4) and further exploited and improved by Cavanagh (2), this indicator electrode is coupled with a reference electrode (a quinhydrone electrode) (1) which gives a steady potential of 0·521 volt, there is then obtained a combination of zero E.M.F. at the end-point of the titration.

The E.M.F. of the combination

$$\underset{a}{\text{Ag, AgCl, Cl}'}\,||\,\underset{b}{\text{H}^+\text{, Quinhydrone, Pt.}}$$

is given by the formula

$$E = E_a^0 - \frac{RT}{nF}\ln\frac{1}{[\text{Cl}']} + E_b^0 - \frac{RT}{nF}\ln\frac{1}{[\text{H}^+]}.$$

At 25° C. this reduces to

$$E = \cdot 4745 - \cdot 0591 \log\frac{1}{[\text{Cl}']} - \cdot 0591 \log\frac{1}{[\text{H}^+]},$$

and at the end-point

$$E = \cdot 4745 - \cdot 2955 - \cdot 0591 \log\frac{1}{[\text{H}^+]}.$$

If the end-point is to be indicated by zero E.M.F., then a solution is chosen such that the value of

$$\cdot 0591 \log\frac{1}{[\text{H}^+]} = \cdot 179.$$

A buffer solution of pH 3·03 gives the desired conditions. At the beginning of a titration when the [Cl′] is greater than 10^{-5} the quinhydrone electrode is the positive pole, and as the titration proceeds the E.M.F. of the combination decreases until at the theoretical end-point it is zero. When the [Cl′] is less than 10^{-5} (as it is when the end-point is passed) the quinhydrone electrode now becomes the negative pole, or in other words, the current reverses direction. This reversal of direction takes place within the range of one drop of the N/35·5 silver nitrate solution used in this laboratory.

Cavanagh was the first to use a quinhydrone electrode as a reference electrode. He also used the Ag-Ag Cl electrode in place of the silver electrode of Müller. He, however, set up the two half-elements in the same solution and so formed a cell without liquid junction potentials. This practice cannot be adopted for soils on account of their varying reaction and buffer capacity. By setting up the quinhydrone cell outside the system, and making internal connection through a salt bridge, this difficulty is overcome and any liquid junction potential is reduced to a negligible magnitude.

The course of an actual potentiometric titration as outlined above on a soil suspension (No. 806) is shown in Fig. 1. As Müller pointed out, in practice it is not necessary to use a potentiometer at all since we are concerned only with the point at which the current reverses its direction —thus indicating the end-point of the titration. A galvanometer is therefore used to indicate this point.

DESCRIPTION OF APPARATUS.

The apparatus used is represented diagrammatically in Fig. 2. The Ag-Ag Cl electrode *A* and the quinhydrone electrode *B* are connected through a tap-key *C* to a moving coil pointer galvanometer *D*.

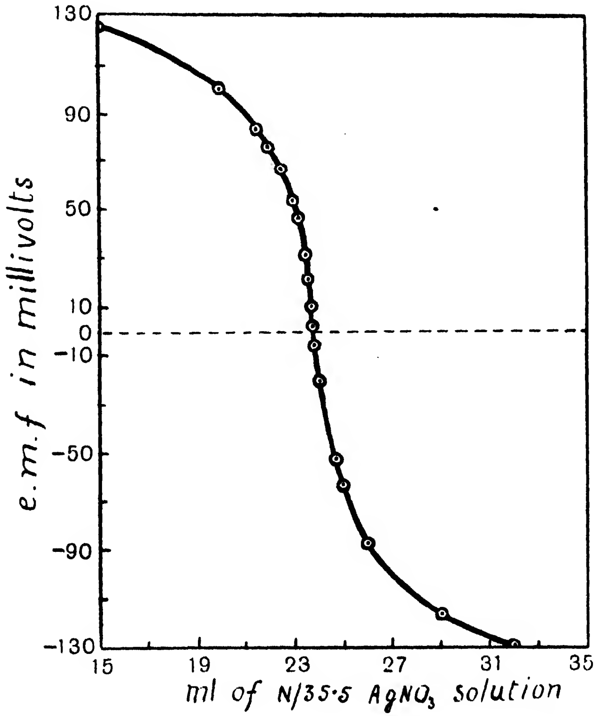

Fig. 1. Showing the potentiometric titration of soil No. 806 with standard silver nitrate solution. The end-point occurs at 23·82 ml. corresponding to 0·595 % chlorine in the original soil.

Internal connection of the two half-elements is effected by means of a 3 per cent. agar-saturated KNO_3 salt bridge *E*. The sensitivity of the galvanometer should be such that it will give a deflection of one division per micro-amp.

If the ends of the agar bridge are close to the platinum and Ag-Ag Cl electrodes, the resistance of the whole system is such that a galvanometer of the above sensitivity can be used directly with soils without an external resistance.

If a more sensitive galvanometer is used, it may be necessary to

insert an adjustable resistance in series with the galvanometer to reduce the deflection at the beginning of the titration.

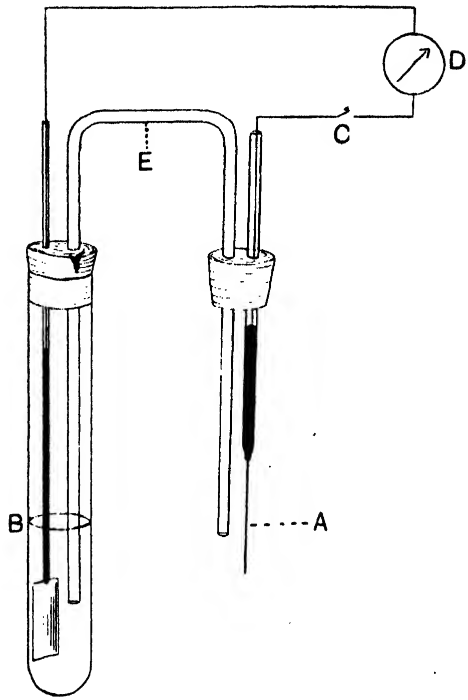

Fig. 2. Apparatus for the electrometric determination of the chloride content of soils.

PREPARATION OF THE ELECTRODES.

The Ag-Ag Cl electrode is prepared by sealing a piece of silver wire 1 mm. in diameter into a piece of glass tubing and completing the seal with a little shellaç. Electrical connection is then made through a mercury contact in the usual way. The wire is then coated over a length of 2 or 3 cm. with silver chloride by making it the anode in the electrolysis of a dilute (0·1 to 0·01 normal) solution of an alkali chloride for one hour. A current density of about 2 to 3 milliamps per sq. cm., as recommended by Cavanagh, drawn from a lead accumulator by inserting a suitable resistance was found to be satisfactory. The electrode, when freshly prepared, is coloured brown. When not in use it should be stored in a test-tube of distilled water in a dark corner. The life of an electrode is at least two months.

When in use the electrode comes to equilibrium almost instantaneously, as was observed during the potentiometric titration. When very near the end-point it should be allowed to stand in the solution three seconds before depressing the tap-key. If it is preferred, the silver wire need not be sealed into glass, but may be connected directly to the lead to the galvanometer by means of a binding screw.

The quinhydrone electrode is made up in the usual way by adding about 0·05 gm. quinhydrone to about 12 ml. of a buffer solution of reaction between *p*H 3·0 and *p*H 3·3, contained in a test-tube of about 25 ml. capacity.

The buffer solution must of course be reasonably free from chloride. The direction of Clark and Lubs (3) may be followed in preparing the buffer solution, if sulphuric acid is substituted for hydrochloric acid. Calculation shows that in order to get the current to reverse at the theoretical end-point, the *p*H of the buffer used should be 3·03 if the titration is carried out at 25° C. and 3·3 if carried out at 16° C. Calculation also shows that if at 25° C. a buffer of *p*H 3·3 is used, the current will reverse direction when the [Cl′] is $1{\cdot}9 \times 10^{-5}$ instead of 1×10^{-5}. If the working volume is 50 ml., this means that the end-point as observed occurs too early by 0·018 ml. of $N/35{\cdot}46$ $AgNO_3$ solution, that is the titre will be about half a drop too little. It is therefore immaterial whether a buffer of *p*H 3·3 or one of *p*H 3·0 is used; further, all errors due to variation of temperature between 16° C. and 25° C. fall within half a drop of the titrating solution and are consequently negligible.

PROCEDURE.

Four grams of the soil are weighed out into a 150 ml. pyrex beaker. About 50 ml. of distilled water are added from a measuring cylinder and the suspension is shaken by hand for a few seconds, and allowed to stand for about five minutes before commencing the titration. The beaker is then raised so that the Ag-Ag Cl electrode and the agar tube dip into the suspension, and the tap-key is depressed. The initial deflection gives a rough idea of the amount of $AgNO_3$ required.

The titration is now commenced and the above procedure is repeated after each addition of reagent. Vigorous stirring with a glass rod after each addition is necessary. The pointer reverses direction within the range of half a drop.

When the chloride content is low, say less than 0·05 per cent., it is advisable to take 10 gm. of soil.

The ratio of soil to water has no effect on the amount of chloride

brought into solution as the same value was obtained for a 1 : 5 ratio as for a 1 : 12·5 ratio with soils of high (0·5 per cent.) and low (0·002 per cent.) chloride content.

It is almost impossible to overshoot the end-point as warning of its approach is given by the pointer. If, however, this is done, it is a simple matter to back titrate with (say) $N/35{\cdot}46$ KCl.

Standardisation of the system.

The whole system may be standardised at the beginning and checked at the end of each day by titrating any convenient quantity of hundredth molar KCl diluted to about 50 ml. with distilled water. The author has obtained the theoretical titre, within the accuracy of burette manipulation, with such regularity that it would seem unnecessary to standardise the system. As, however, the apparatus may be set up and the standardisation completed in ten minutes, this procedure is recommended. Further, if it should so happen that the reaction of the buffer solution is appreciably different from the limits discussed above, then the current reversal occurs not at the theoretical end-point, but at some other, but definite value of the [Cl′] and an absolute correction can be applied.

Experimental results.

In Table I is set out a comparison between the values determined electrometrically, on the one hand, and by the indicator method, on the other. The values determined by the latter method were taken from the records of the Waite Institute, and were obtained by employing the titration and filtration technique recommended by Prescott and Piper (5). The soils were chosen from the Murray Mallee and the Renmark Irrigation Area, soil series noted for their varying salt content. The same values were obtained whether the soil suspension was titrated after standing for five minutes or overnight. In practice thirty-five titrations were carried out in four hours. The figures given in the second column are the means of at least three titrations. The maximum difference between separate determinations of the same soil and the mean is 0·10 ml. This difference, which occurred in two cases only, and other differences which are smaller but lie outside the experimental error of the titration, must be due to sampling error in the laboratory, which is, therefore, very small. In most cases, the differences fall within half a drop of the titrating solution and, therefore, within the experimental error of the titration.

Table I. *Comparison of electrometric and indicator methods for determining the chloride content of soils.*

Soil no.	% Cl Electrometric	% Cl Indicator	Soil no.	% Cl Electrometric	% Cl Indicator
303	·001	·002	955	·070	·073
310	·002	·002	293	·077	·080
964	·002	·003	299	·089	·089
311	·0045	·005	353	·096	·096
963	·005	·004	956	·111	·114
975	·017	·017	324	·148	·152
953	·019	·021	327	·155	·158
298	·019	·020	957	·173	·171
954	·041	·041	958	·248	·251
976	·042	·046	644	·412	·417
315	·047	·047	806	·593	·613

The soils of high salt content flocculate and leave a clear supernatant liquid after settling for some hours. A set of four such soils was taken and the chloride content determined: (*a*) on the suspension as described above; and (*b*) by shaking in an end-over-end shaker for one hour, allowing to settle and pipetting off aliquot portions of the clear supernatant liquid and titrating electrometrically.

The results, together with the values determined by the indicator method, are set out in Table II.

Table II. *Comparison of values obtained by titrating* (*a*) *soil suspension,* (*b*) *clear supernatant liquid.*

	% Cl		
	Electrometric titration		
Soil no.	(*a*) Suspension	(*b*) Supernatant liquid	Indicator titration
612	·300	·297	·292
805	·537	·530	·547
847	·566	·566	·580
686	·778	·780	·757

It is clear that a titration may be carried out on the suspension itself and the result will give the chloride content of the soil with a high degree of accuracy. Where the electrometric and indicator methods give different values, the electrometric method should be regarded as the standard by reason of its being absolute and subject to no personal error other than that incurred in reading the burette. The agreement between the two methods is, however, well within the field sampling error and in consequence the chief advantage of the new method is the great saving of time and the easier detection of the end-point.

The soils tabulated in Tables I and II contain a low percentage of organic matter. To test the applicability of the method in the case of soils of high humus content, a soil from the reclaimed swamps of Murray Bridge, South Australia, was examined. The percentage of Cl was determined electrometrically after standing with water for five minutes to be 0·069. On standing overnight, however, more chloride went into solution and the percentage of Cl was found to be 0·074. On standing for longer periods, no more chloride went into solution. The indicator method on the filtered extract gave the value 0·071 per cent. Cl. It is evident that if no higher accuracy is desired than can be obtained by the indicator method even this type of soil can be titrated after standing for ten or fifteen minutes. If greater accuracy is desired, the soil suspension should be allowed to stand overnight, to allow mechanically held chlorides to go into solution.

The author wishes to express his thanks to Prof. J. A. Prescott, who suggested the possibility of applying this method to soils, for the helpful interest taken in the work and for assistance in selecting the soils.

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THE COBALTINITRITE (VOLUMETRIC) METHOD OF ESTIMATING POTASSIUM IN SOIL-EXTRACTS.

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THE platinichloride and perchlorate methods for the estimation of potassium are dropping into disfavour amongst soil chemists. When applied to small-quantity work, under the somewhat severe conditions of a soil-analysis, they are beset with difficulties. They may lead at times to results of quite the wrong order of magnitude. Gross errors of this kind are conceivably resident in much of the extant data upon soil potassium, where the work has been done in uncritical faith in these methods. Such errors can indeed be avoided, but only at the expense of much manipulation, itself forbidding expectation of a nice accuracy at the end of it. It is becoming a general opinion that these methods are a discouragement rather than an aid to work in this field.

The requirements which a suitable method must meet are fairly exacting. It must enable small differences to be picked out between quantities themselves small, such as occur in citric acid or ammonium chloride extracts; quantities frequently of the order of between 5 and 10 mg. K_2O. For such purposes the systematic error of the estimate (losses during separation, or gains by retention of accompanying foreign substances) must be brought well below 0·5 mg. It must also be possible to achieve accuracy of mean values, by replication, without undue expense in time or material.

The third of the chief available methods, viz. the cobaltinitrite method, in its volumetric form, fulfils, in the main, the above requirements. It is not yet, however, generally employed by soil chemists as a piece of standard practice. No sufficient body of successful experience with it is on record, for it to be widely used with confidence. The purpose of this paper is to add some data upon the method to the scanty existing records, towards remedying this position. Variants of the cobaltinitrite method have indeed been in use here and there by agricultural chemists for years. Where, then, the procedure here described (p. 547) differs in detail from other versions of the method in use elsewhere, it may not necessarily be that the differences are improvements. The object is

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rather to call attention to the soundness of the method in at least one of its forms, than to put forward a new modification of it.

The principle of the method is well known. Potassium sodium cobaltinitrite, of the approximate formula $K_2NaCo(NO_2)_6, 6H_2O$, is precipitated by evaporation with sodium cobaltinitrite solution, or an equivalent mixture of reagents. The precipitate is finally oxidised in boiling standard permanganate. A procedure on these lines was described by Drushel (1) (1907–8), who assumed the above formula to be exact, and calculated results from an equation, using the factor

$$1 \text{ c.c. } N/10\ KMnO_4 = 0{\cdot}000856 \text{ gm. } K_2O.$$

Dependence of relative estimate upon amount of potassium taken. Mitscherlich and Fischer (2) (1912), examining the validity of this factor, showed that an estimate of a known small amount of potassium was liable to be relatively higher with higher relative quantities of precipitating reagents, owing to changes in the composition of the precipitate in the direction of containing more sodium, and hence more oxidisable NO_2 per unit of K. They defined limits within which the ratio of precipitants to precipitate (Fällungsfaktor) should fall. This serious disadvantage was tackled by Christensen and Feilberg (3) (1920) by precipitating from a solution saturated with sodium chloride, thus attempting to stabilise the composition of the precipitate. These authors employed a new empirical factor in place of the theoretical one. Christensen and Feilberg's own data show that even though their procedure made the percentage recovery of known amounts nearly independent of the amount to be estimated, the effect was still observable over a range of 0·5 to 15 mg. K_2O (in round figures) for fixed quantities of precipitating reagents.

Table I. *Different quantities of* K *with fixed quantities of precipitating reagents; Christensen and Feilberg's procedure, unmodified.*

Relative quantities of K taken	Mean percentage recovery	Number of experiments
4 (= about 13 mg. K_2O)	89·3	8
2	93·9	12
1	97·0	4

The point is of such importance that it was examined at the outset of the present work. Quantities of pure KCl were taken, varying relatively to the fixed quantities of reagents used by the above authors (viz. 3 c.c. 10 per cent. $CoCl_2$, 5 c.c. 10 per cent. $NaNO_2$, 5 c.c. saturated NaCl), in the proportions shown in Table I. Column 2 of the table

records the mean percentage estimates. The factor used, and the procedure throughout, were those of Christensen and Feilberg.

In an endeavour to postpone the operation of the effect, here clearly demonstrated, to a range of quantities above those met with in the kind of work in mind, the quantities of reagents were increased in subsequent work to 10, 15 and 10 c.c. of the above reagents respectively. The success attained may be judged from Tables II, III and IV. Tables II and III embody results yielded by the method in this form (and with other modifications included in the description on p. 547) upon certain plant-ash solutions which offered favourable material. Permanganate titrations are given to show the size of the quantities estimated, and these are calculated also to per cent. of K_2O in the original samples, to show the practical significance of the errors involved. Table II concerns analyses of solutions of mixed alkali sulphates derived from six samples of hay. The quantities were varied as 1 : 2½, with fixed quantities of reagents. In Table III there are four analyses of each of two "mixed sulphates" solutions from separate portions of a single sample of silage. The quantities were varied as 1 : 8, reagents always as above.

Table II. *Different quantities of* K *with fixed quantities of reagents; plant-ash solutions.*

1	2	3	4	5	6	7
	Titrations in c.c. $N/20$ $KMnO_4$		*For strict proportionality col. 3 should read*	Errors of col. 3 as % of col. 4	Cols. 2 and 3 expressed as % of original plant-substance	
Solution	10 c.c. taken	25 c.c. taken			Col. 2	Col. 3
	c.c.	c.c.	*c.c.*	%	%	%
A	16·0	40·9	*40·0*	+2·25	1·67	1·71
B	27·1	68·95	*67·75*	+1·75	1·41	1·44
C	18·4	48·1	*46·0*	+4·8	1·99	2·08
D	35·55	90·05	*88·9*	+1·3	1·83	1·86
E	33·35	83·35	*83·4*	−0·1	1·68	1·68
F	16·95	41·95	*42·4*	−1·0	1·73	1·72

Table III. *Different quantities of* K *with fixed quantities of reagents.*

1	2	3	4	5	6	7
Quantities taken for analysis	Titrations in c.c. $N/20$ $KMnO_4$		Errors as % of "5 c.c." values		Cols. 2 and 3 expressed as % K_2O in dry sample	
Solution	*G*	*H*	*G*	*H*	*G*	*H*
5 c.c. (=about 4 mg. K_2O)	10·05	10·3	—	—	1·515	1·55
10 c.c.	20·15	20·55	+0·3	Nil	1·52	1·55
20 c.c.	41·85	42·1	+4·3	+2·6	1·58	1·59
40 c.c. (=about 32 mg. K_2O)	82·7	82·25	+3·0	Nil	1·56	1·55

The two tables cover a range of from 4 to nearly 40 mg. K_2O (10 to 90 c.c. $N/20$ $KMnO_4$), and show no consistent errors attributable to the effect under investigation. If present, these should be negative in sign for the larger quantities. The significant differences are, however, all positive. The largest error (solution *C*, Table II) represents 0·36 mg. in 7·6 mg.

Tables II and III do not concern the *absolute* correctness of the amounts of potassium reported. Table IV does so. It summarises 31 analyses made upon known quantities of pure KCl, at odd times over more than three years. The samples of KCl used differed from time to time, as did other relevant circumstances. Although therefore these data are not strictly comparable *inter se*, as are those of Tables II and III, they do afford a test of the degree to which the effect under discussion shows up amidst the casual errors of performance of the same procedure in different circumstances.

Table IV. *Summary of trials with pure* KCl.

Quantities taken for analysis	Number of experiments	Mean estimate of each quantity as %
Pure KCl = about 6 mg. K_2O	10	101·3 ± ·8
= about 10 mg. K_2O	12	99·8 ± ·65
= about 12·5 mg. K_2O	4	99·2 ± ·5
= about 20 mg. K_2O	4	99·2 ± ·8
= about 30 mg. K_2O	1	95·8

Mean of the 31 results (irrespective of quantity taken) = 100·0 % ± ·43
Standard error (single result) = ± 2·38 %
Highest single estimate = 104·0 %
Lowest single estimate = 95·8 %

Though a dependence of estimate upon quantity estimated may seem to be just distinguishable (cf. the means of 101·3 for 6 mg., and 99·2 for 12 and 20 mg.), the differences are not significant. It is clear from the data of these tables that a single estimate of from 4 to 40 mg. K_2O, made with the fixed quantities of reagents above specified, may be relied on as to absolute magnitude safely within 5 per cent. The possibility of high results with small quantities, and *vice versa*, should not, however, be lost sight of when applying the method to quantities outside the above range. For example, Morgulis and Perley (4) (1928), dealing with blood-plasma analyses by this method, advise calibration against pure potassium solutions for work outside their usual range of 0·2 to 0·3 mg., owing to the prominence of the effect in their procedure[1].

[1] From the abstract seen it does not appear whether these authors adopt the principle of using a large excess of precipitants in order to anticipate variations in the sodium-content of the precipitate.

In extreme cases this may also be necessary in the present larger-scale procedure.

Influence of calcium and magnesium salts, and of sulphates. The above data and conclusions refer to the simple case of solutions containing only the alkali metals. In the gravimetric methods, imperfect removal of alkaline earth sulphates leads to high results, and elimination of sulphate-ion leads, or may lead unless there is considerable dilution at this stage, to losses of potassium in the $BaSO_4$ precipitate. The present method, depending on the titration of nitrite-groups, is independent of the presence of sulphates. The point is illustrated by the data of Tables II and III, and of Table VI. The question of Ca and Mg, whether as chlorides or sulphates, is already covered by existing published data, *e.g.* Christensen and Feilberg's results upon artificial kainit- and sôil-solutions (*loc. cit.* (3)). It is, however, often of such great convenience to conduct the potassium analysis directly upon an HCl-extract containing other cations and anions, rather than in its usual place (in a complete analysis) upon the mixed sulphates, that some relevant data are presented here. Table V is self-explanatory. Table VI summarises figures selected from ash-analyses of six feeding-stuffs.

Table V. *Effect of* Ca *and* Mg*; pure* KCl *solutions.*

Quantities taken for analysis	Additions made	Estimates as % of amounts taken
$KCl = 6{\cdot}315$ mg. K_2O	$CaCl_2 = 3{\cdot}5$ mg. CaO	101·3
	$= 7{\cdot}0$ mg. CaO	99·5
	$= 14{\cdot}0$ mg. CaO	102·3
	$= 28{\cdot}0$ mg. CaO	99·6
$KCl = 6{\cdot}315$ mg. K_2O	$MgCl_2 = 2{\cdot}5$ mg. MgO	102·9
	$= 5{\cdot}0$ mg. MgO	96·1
	$= 10{\cdot}0$ mg. MgO	97·8
	$= 20{\cdot}0$ mg. MgO	96·2
$KCl = 7{\cdot}89$ mg. K_2O	$CaCl_2 = 14$ mg. CaO, + $MgCl_2 = 10$ mg. MgO, added to each	104·0
$= 6{\cdot}315$ mg. K_2O		104·1
$= 4{\cdot}74$ mg. K_2O		103·5
$= 3{\cdot}16$ mg. K_2O		107·6
$= 1{\cdot}58$ mg. K_2O		115·9

In Table VI, column 2 gives results upon HCl-solutions after removal of silica only, and column 3 gives results upon mixed sulphates derived from similar aliquots of the same solutions after removal of sesquioxides, phosphates, and alkaline earths. For direct analysis for K upon the HCl-extracts, the aliquots were merely evaporated to dryness, taken up in water, and the cobaltinitrite reagents added immediately without filtering. Except for the last two items of Table V (which, being estimates of quantities on the edge of the normal range, may be influenced by the "precipitation-factor" effect discussed in the previous section),

the results exhibit no dependence on the amounts of Ca or Mg present, nor on the presence of sulphate or phosphate.

Table VI. *Effect of* Ca, Mg, PO_4*; plant-ash solutions.*

1	2	3	4	5	6	7
	Estimate on HCl solution, containing K, Na, Ca, Mg, PO_4, SO_4. c.c. $N/20$ $KMnO_4$	Estimate on "mixed sulphates" solution containing K and Na only. c.c. $N/20$ $KMnO_4$	Cols. 2 and 3 expressed as % of K_2O in sample		Two other independent estimates on the same samples (HCl solutions)	
			Col. 2	Col. 3		
Sample	c.c.	c.c.	%	%	%	%
J	58·85	57·4	3·14	3·23	3·05	3·16
K	51·1	51·3	2·44	2·45	2·39	2·40
L	62·95	61·1	3·40	3·30	3·28	3·18
M	34·9	33·3	1·89	1·80	1·85	1·85
N	28·0	28·85	2·36	2·43	2·44	2·37
O	24·8	22·75	1·27	1·16	1·27	1·26

Table VI includes, in all, four independent estimates upon the same set of samples, and will demonstrate the degree of reproducibility of results in such work.

Empirical factor for conversion of $KMnO_4$ *titrations into weights of* K_2O. It has been mentioned that Christensen and Feilberg proposed an empirical factor for calculation of results. Its value was 0·000805 per c.c. $N/20$. This factor, however, used with their procedure, has given low results. In 24 trials with pure KCl (not comprised in the summary in Table IV), the mean percentage recovery of expected amounts was 94·5 per cent. ± 0·5. The theoretical factor would have given 100·5 per cent., and is, therefore, more appropriate. This same theoretical factor is also found valid by other workers using similar versions of the method, *e.g.* by Leitch Morris (5) (1923), by Morgulis and Perley (4) (*loc. cit.*), and by the Rowett Institute staff (private communication[1]). The factor 0·000830, recommended for use with the version of the method here described and employed for all the data of this paper except those of Table I, therefore, requires explanation.

Several minor differences exist between the present form of the method and other forms described elsewhere for which the theoretical factor is valid. The significant one in this connection is that oxidation is conducted in acidified permanganate instead of in neutral, as is more usual. This appears to have the effect of diminishing the equivalent value of the permanganate, so that more is required for a given weight of potassium, and a smaller factor is needed. The data of Table IV would have a mean of 103·1 per cent. ± 0·44 with the theoretical factor, whose

[1] By courtesy of Mr W. Godden, 1927.

theory is evidently slightly at fault in the conditions of these analyses. The factor appropriate to the conditions is $\frac{100}{103 \cdot 1} \times 0 \cdot 000856 = 0 \cdot 000830$.

The writer holds no brief for this factor except in its context, as yielding 100 per cent. results with the procedure described; and it is put forward without prejudice to the validity of the theoretical factor for other forms of the method.

The diluted permanganate used for the oxidation should be at or very near boiling-point when the precipitate is introduced. It is better to heat directly over a flame than to suspend in a water-bath. Definitely different (low) results are obtained if the precipitate is added to *cold* permanganate, which is then brought to the boil, as, for instance, in the procedure of Haff and Schwartz (6) (1917). Six trials on these lines gave a mean recovery of 78 per cent. of the expected amounts. If such a variant of the method is adopted, a new empirical factor is required, to correspond with the altered conditions.

DESCRIPTION OF THE METHOD.

The particular form of the cobaltinitrite method which has yielded the results tabulated in this paper (except those of Table I) is described below.

Reagents required. $NaNO_2$, 100 gm. per litre; NaCl, saturated solution; $CoCl_2$, $6H_2O$, 100 gm. per litre; acetic acid, 100 gm. per litre; Na_2SO_4, 25 gm. per litre; glass dust, passing 100-mesh sieve, in suspension in water; standard $KMnO_4$, conveniently $N/20$; standard oxalic acid, conveniently slightly $> N/20$, made up to contain 50 c.c. pure H_2SO_4 per litre[1].

It should not be assumed that any of the reagents are free from potassium until satisfactory "blanks" have been obtained. The most likely reagent to be under suspicion is sodium nitrite.

The solution prepared for analysis should be neutral and free from ammonium salts and organic matter. It is undesirable to work alongside other workers who are using ammonia. Aliquots representing up to 50 mg. K_2O (requiring about 120 c.c. $N/20$ $KMnO_4$) may be taken for the quantities of reagents specified, but from 5 to 25 mg. is most convenient.

The bulk is reduced to about 10 c.c. in a 3-inch glazed evaporating-dish. 10 c.c. saturated NaCl, 10 c.c. 10 per cent. $CoCl_2$, and 15 c.c.

[1] Pure aqueous oxalic acid solutions of this normality require frequent checking. As described, they are more permanent. See Treadwell and Hall, *Analytical Chemistry* (John Wiley & Co.) II, 599.

10 per cent. $NaNO_2$ are added in the order stated and mixed with a short glass rod, which is left in the basin. The mixture is evaporated on the steam-bath to stiff pasty condition or hard dryness, being well stirred from time to time, especially towards the end of the evaporation, to work the crystalline crusts down into the liquid. After cooling[1], 10 c.c. 10 per cent. acetic acid is run in and the mixture well stirred to assist solution of the excess reagents and NaCl. After standing a quarter of an hour, 10 c.c. of water is stirred in, and the mixture is filtered through a small Gooch crucible, with suction. The filter[2] consists of a disc of No. 40 Whatman paper, pressed well down at the edges, and covered with a layer of the finer particles from a suspension of glass dust in water. The precipitate is washed by decantation with $2\frac{1}{2}$ per cent. sodium sulphate, transferred to the crucible, and washed six or eight times with the same wash-liquid[1]. Total washings need not exceed 25 c.c.

Allowing for an excess of at least 10 c.c., a measured quantity of $N/20$ permanganate is diluted and brought to the boil. 10 c.c. of dilute sulphuric acid is added roughly from a burette, and the solution again boiled and removed from the flame. The precipitate in its crucible is now immediately added and stirred well round, and the beaker covered and set aside for ten minutes. After two or three minutes hydrated MnO_2 separates, and if left longer than 15 to 20 minutes this may be slow in redissolving later. A measured volume of standard oxalic acid, sufficient to give a perfectly clear solution, is now added, the crucible removed, and the excess titrated with the same $N/20$ permanganate. The end-point is quite sharp.

A complete "blank" analysis should be done (better two or three) for each new set of reagents, and may with advantage be repeated from time to time during a long series of analyses.

Factor. The corrected volume of permanganate is converted to its equivalent of potassium by the factor 0·000415 gm. K_2O per c.c. $N/20$ $KMnO_4$ (see discussion on p. 546).

The method applied to soil-extracts.

Citric acid extracts. Table VII illustrates the kind of results which the method as above described will yield upon citric acid extracts. The nine trials with Soil *A* and the six with Soil *B* were done upon aliquots (of 500 c.c. each) of uniform large bulks of 1 per cent. citric extracts of the

[1] If the analysis has to be interrupted, it should be at these points only.

[2] A 12 mm. disc accounts for some 0·25 c.c. $N/20$ $KMnO_4$ in a "blank." There is possibly room for improvement here. The filter as described, however, gives clear filtrates, is rapid, and has advantages of convenience.

respective soils, prepared in the usual manner. For Soil *A*, organic matter was removed by evaporation with nitric acid, followed by gentle ignition. The potassium-analysis was done directly on a water-extract from the finely-powdered residue. For Soil *B*, organic matter was removed by ignition, without nitric acid, followed by removal of silica, a second ignition, and water-extraction as before. Of these two treatments, the first avoids possibility of losses of K by volatilisation during the prolonged ignitions. The second avoids the difficulty of controlling effervescence, with its spray-losses, during evaporation with nitric acid. The first procedure is far the shorter.

Table VII. *Citric acid soil-extracts.*

Solution	K_2O found mg.	Mean of three trials mg.	Added K_2O recovered mg.	%
Soil *A* (heavy clay):				
Citric extract with no addition	4·27 4·39 4·35	4·34	—	—
Citric extract with added KCl = 5·0 mg. K_2O	9·28 9·01 9·32	9·20	4·86	97·3
Citric extract with added KCl = 25·0 mg. K_2O	28·64 28·34 28·04	28·34	24·0	96·0
Soil *B* (moderately heavy subsoil):				
Citric extract with no addition	2·86 3·08 2·90	2·95	—	—
Citric extract with added KCl = 6·31 mg. K_2O	8·98 8·87 9·22	9·02	6·07	96·2

The table, which is self-explanatory, shows little advantage in either of the two preliminary treatments over the other, as judged by uniformity of the triplicates, or by percentage recovery of known added quantities of K. There is no evidence of serious losses of potassium or inaccuracies of other kinds.

Cobaltinitrite and perchlorate analyses compared. As a matter of intrinsic interest, and as supporting the statements made in the introductory paragraph of this paper, there are presented in Table VIII the results of a parallel series of analyses made upon the same bulk extract of Soil *B*, using the perchlorate method. Up to the point of adding perchloric acid, the procedure was identical with that already described for Soil *B*. The question of sulphates then arose. W. A. Davis (7) (1912) held that "in dealing with practically all soil extracts, treatment with baryta is unnecessary, as the proportion of sulphates is generally very

small." His data, however, are insufficient to support such a conclusion. Dodd (8) (1924) considered the above-quoted dictum quite unsound. One may only hope that it has not led to the accumulation of too many unsound results. In order to put its validity to test in the present case, sulphates were *not* removed from the solutions, but the effect of the omission was subsequently studied by examination of the perchlorate precipitates.

Table VIII. *Perchlorate analyses, citric extracts, parallel with the cobaltinitrite analyses of Table VII, Soil B.*

Solution	Sulphate not removed: Weight of supposed $KClO_4$ precipitate gm.	Sulphate not removed: K_2O calculated mg.	Sulphate not removed: Added K_2O recovered mg.	$CaSO_4$ found in precipitate gm.	Corrected for sulphate found: K_2O found mg.	Corrected for sulphate found: Added K_2O recovered mg.
Soil *B*						
With no addition						
	·0240	8·15	—	·0107	4·5	—
	·0298	10·1	—	·0140	5·4	—
	·0208	7·1	—	·0076	4·5	—
With added KCl = 6·31 mg. K_2O						
	·0398	13·53	5·1 = 81 %	·0097	10·2	5·4 = 86 %
	·0414	14·07	5·6 = 89 %	·0115	10·2	5·4 = 86 %
	·0388	13·18	4·7 = 75 %	·0098	9·9	5·1 = 80 %

Mean figures are not worth reporting on such results as those of Table VIII, but the perchlorate estimates of the K_2O-content of the original solution, viz. 8·15, 10·1 and 7·1 mg., should be compared with the cobaltinitrite estimates on the same solution, viz. 2·86, 3·08 and 2·90 mg. The "perchlorate" recovery of 6·3 mg. of added potassium (75, 81, 89 per cent.) should also be compared with the "cobaltinitrite" recovery (94, 95, 99 per cent.). The perchlorate precipitates contained sulphate. But even after correcting for its amount (as $CaSO_4$, ascertained by precipitation at considerable dilution as $BaSO_4$), the results remain unsatisfactory. The figures well illustrate the uncertainties of small-quantity potash work on soils, if methods and data are not examined critically. In judging whether to accept the perchlorate or the cobaltinitrite mean figure as the truth about the potassium-content of the above soil-extract, both agreement of replicate analyses, and the check on a known amount of potassium, would lead to rejection of the perchlorate estimate.

Ammonium chloride extracts. These are more difficult to handle than citric extracts, since some 27 gm. of ammonium chloride (contained in the 500 c.c. *N* NH_4Cl used for leaching) have to be removed before the

10 mg. or so of exchangeable potassium can be precipitated. The difficulties are irrelevant to the subject-matter of this paper except in so far as they affect the cobaltinitrite analysis itself. Provided that removal of NH_4Cl has been complete, there is no likely cause of disturbance. The presence of manganese, which occurs frequently in ammonium chloride extracts, has been shown to have no effect in trials in which pure KCl solutions containing manganous salts have been analysed.

It seems nevertheless worth mention that trials with ammonium chloride extracts on the same lines as those reported for citric extracts have given unsatisfactory results. Some such are given in Table IX.

Table IX. *Ammonium chloride soil-extracts.*

	Different estimates on similar aliquots of an NH_4Cl extract	Examples of % recovery of known added amounts of K
Soil *C* (sandy loam)	6·13, 5·56, 5·95 mg. K_2O	77, 88, 85 %
Soil *D* (chalk soil)	9·89, 9·17, 9·26, 8·12 mg. K_2O	61, 77 %

The cause of the gross inaccuracies of the above table is not fully known, and the results are offered tentatively, to call attention to the possibility of serious error in exchangeable potassium determinations involving the removal of large quantities of ammonium chloride. It is not suggested that the above results are typical—merely that such results, due to other causes than careless handling, do occur. Careful checking of "exchangeable K" figures sought by this method is evidently necessary. A neat and cleanly means of destroying large quantities of ammonium salts, when an appreciably volatile constituent is to be estimated in the residue, is much to be desired.

SUMMARY OF CONCLUSIONS.

The cobaltinitrite method, in the volumetric form here described in detail, enables known amounts of potassium to be accounted for quantitatively, independently of the presence of alkaline earth sulphates, or phosphates, provided that the amount of potassium is not varied over too great a range. The factor 0·000830 gm. K_2O per c.c. $N/10$ $KMnO_4$ suits the procedure described over a range of about 3 to 50 mg. K_2O. Outside this range, or for highly accurate work within it, it may be desirable to calibrate the method.

The method may be applied, in plant-ash analyses, indifferently to the original extracts containing other bases and phosphates, or to the mixed sulphates weighed for sodium and potassium together. It is

applicable to small-quantity work upon soils with greater exactness and speed than is the perchlorate method. Citric acid extracts can be handled, with a relatively short manipulation, to give satisfactory results.

Some analyses of ammonium chloride extracts have been unsatisfactory, and attention is called to the desirability of setting exchangeable potassium determinations upon a firmer analytical basis, by investigation of methods of freeing the extracts from ammonium salts.

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THE EFFECT ON LACTATION OF THE LENGTH OF THE PRECEDING CALVING INTERVAL AND ITS RELATION TO MILKING CAPACITY, TO AGE AND TO OTHER FACTORS OF INFLUENCE.

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A number of studies have been published in recent years dealing with what may be called the minor factors of environment and their influence on milk yield. In particular there is the exhaustive work of Sanders (1, 2, 3, 4).

Most of these publications trace a relation between the length of the period between calving and fruitful service and the yield in the *lactation that accompanies*, and generally they go to show that within fairly wide limits the longer the average calving intervals the higher the yield in life.

On the other hand one or two writers seem to suggest that highest life time milk production can only come by the highest frequency of pregnancies. Eckles (5) contends that a long interval benefits current lactation but depresses the one to follow, while Gaines (6) states that "frequent calving is essential to maintain the mammary gland at the highest average rate" of secretion. Incidentally Gaines does not find evidence that in cows lactation has a tendency to inhibit ovarian activity.

Conclusions arrived at from experience in India.

In the last 25 years the writer has had several thousand cows under continued observation on the Army Dairy Farms under his control in India, and has given these matters much consideration. In the beginning they appeared unimportant, but as the cattle were improved by selective breeding and by feeding it was presently found that the later lactations did not show the same relative increase over the first as they had previously, and the subsequent study and experiment appeared clearly to indicate that the most important factor governing milk yield, after heredity and diet, is the length of the calving interval that precedes a given lactation, and that, broadly speaking, very short intervals—*i.e.* frequent pregnancies—depress the life time yield, long ones increase it.

This conclusion is in principle the same as taught by the researches of Sanders. This paper does not seek merely to restate it but to draw

attention to aspects of the case which so far as is known have not been discussed in any journal hitherto.

It is submitted first that the essential factor is not the length of interval (or of "S.P.," which determines it) that accompanies a yield, but of the one that precedes it. Secondly that the optimum interval is not a constant, but varies with milking capacity and with age. Third that the calving interval has a strong influence on the effect of length of dry period, and fourth that it has a pronounced bearing on the question of (milking) longevity which has excited so much attention of late (7).

The following "law," laid down to guide the Managers of herds in India referred to above, appears to have worked successfully, namely, that "The optimum calving interval varies directly with milking capacity and inversely with maturity."

SERVICE PERIOD CURRENT OR CALVING INTERVAL PRECEDING?

It will be convenient to designate these respectively S.P. and C.I.P. The writer believes it to be erroneous to ascribe much influence on weekly yield to the length of the service period that accompanies a given lactation. Prolongation of the S.P. will necessarily increase the total yield but its effect on average weekly yield has, it is suggested, still to be determined.

It is true that tables prepared from the statistics of milk recording societies and the like, carried on through consecutive lactations, show an apparent relation of S.P. to weekly yield, but this is believed to be due to the impossibility of avoiding "herd" effect. In one herd cows are kept from the bull, in another they are not; the former give the larger yields but the high yield of the 2nd lactation is due to the long interval during the 1st, of the 3rd to the 2nd and so on.

Similarly a proportion of cows do not conceive during the high levels of lactation and thus automatically run consecutive long intervals—*e.g.* cow Salsette, Table IV—and these are among higher yielders. Such cows if 1st lactations are tabled separately cause a relationship of S.P. and weekly yield to appear, but if their detailed daily yields are examined it is seen that they had reached higher yield levels before the short S.P. cows had been served, and if the same individuals are studied through succeeding lactations the illusion is finally dispelled.

Furthermore it seems that a substantial error creeps in, in another way, if S.P. is aligned with current yield, in that all herds contain cases (few cows of appreciable age escape being so influenced at least once in

life) in which health is injured temporarily and the cow fails to breed. The result is an exceptionally long interval and a low weekly yield accompanying; the table will ascribe the latter not to the poor health but to the extra long interval and the optimum interval is under-estimated in consequence.

RELATION OF OPTIMUM "CALVING INTERVAL PRECEDING" TO MILKING CAPACITY.

From the many thousand life histories of cows on the Army Farms (which are recorded in minute detail) it has been difficult to find a large number fulfilling the conditions imposed, namely, in each case, an unbroken series of at least three lactations—apart from 1st lactations, whose yields are ignored—unaffected by any factors except C.I.P. and D.P., because large numbers of the cattle are moved annually to the hills while diseases such as foot and mouth, contagious abortion, etc. are endemic and there is wide variation in breed characteristics, so that Tables I and II here submitted are made up from the records of only

(*a*) 210 lactations of 52 cows of unimproved native stock, homogeneous in type, forming part of one herd,

(*b*) 378 lactations of 72 cows, product of breeding up from native stock, whose milking capacity is above 5000 lb., selected from four herds. (In all cases a stated yield capacity means that given in the first 300 days after calving unless the number of days is mentioned.)

The method of comparison is first to correct all yields for age using Sanders' figure of conversion, secondly to group the cows according to average length of the preceding interval, and then to take the number of days, from the beginning of the dry period immediately preceding the first of the consecutive lactations to the end of the last lactation, and divide these into the total yield. Admittedly it is rough but no acceptable alternative could be found, as, if total lactation yields between 1st and last calving are compared, there is the difficulty that the last dry period can have no bearing on the milk yield preceding it, while it is not possible to apply corrections for D.P. as this is believed to be more or less a part of the very factor—C.I.P.—which is being studied. It is suggested however that the method adopted is not without merits; a cow's milking life does not begin with her 1st calving but with the preparation for that calving and it finishes with her last drying. A period covering several consecutive lactations from drying to drying gives, therefore, a more representative segment of life than a period from one calving to another.

The number of cows in Table I is rather small, that it is submitted notwithstanding is due to the herd being one of peculiarly homogeneous type, geographically so situated that there has been no crossing, and practically no disease, ever since the writer has known it.

Table I. *Unimproved native cows—low milking capacity—average daily yields.*

Group	*A*	*B*	*C*	*D*
Limits of preceding calving intervals included, days	Under 336	336–356	357–380	Over 380
Number of cows	11	25	12	4
Number of lactations studied	48	106	43	13
Average preceding calving interval, days	329	346	364	405
Average daily yield uncorrected for age	10·4	10·5	10·1	10·0
Average daily yield corrected for age	11·0	11·4	10·8	10·7

It suggests that for low yield cows the pregnancies can hardly be too frequent but it is doubtfully wise to accept that without reserve. As these cows were in no way controlled, it follows that the few whose C.I.P. is long must have been subject to extraneous influence of some sort. All experience of these herds goes to show that cows whose intervals are artificially prolonged respond differently, and the most one can recommend as safely deducible is that there is no substantial advantage to be gained by long intervals, if the cows are of low yield capacity.

If however it is accepted at face value as representing "average cow" and thus comparable with Sanders' results (cf. (1) Table II), it is seen that cows of about 4000 lb. mature standard capacity are suited by average C.I.P. of about 346 days against about 400 days for the 8000 lb. cow.

Incidentally it is remarkable how few of these low yield cows fail to conceive soon after calving. They differ markedly from the higher yield herds in this respect.

In Table II there is a large proportion of cows which were artificially controlled and hence the yields of many of the longer interval groups are less influenced by undiagnosed sickness and the like. Unfortunately the exact proportion is not known as the system of control took much time to establish effectively, and broke down at times with changes of management, etc.

From careful study and comparison with large numbers of other single lactations it is thought to be very fairly reliable in its suggestion that, for cows of 5000–6000 lb. (4·6 per cent. fat) mature standard capacity, the optimum average interval through life is not less than 420 days. Inferentially the 8000 lb. cow requires a still longer interval.

Sanders' Table II (1) suggests only 400 days; but the tables differ in their basis of compilation, as one takes in all cows, and the other is selected to include only healthy normal cows. It is believed that if the material used for Sanders' table could be sifted the optimum figure would be higher.

Table II. *Cows produced by selective breeding—relatively high milking capacity.*

Group	*A*	*B*	*C*	*D*
Limits of preceding calving intervals included, days	Under 343	343–365	366–395	Over 395
Number of cows	12	22	21	17
Average preceding calving interval, days	334	356	376·5	429·7
Equivalent preceding service period	54	76	96·5	149·7
Average daily yield uncorrected for age	13·1	14·3	15·0	14·8
Average daily yield corrected for age	13·2	14·5	15·0	15·3

ALTERNATIVE METHODS OF ASSESSMENT.

The statistician restricted to M.R. Societies' records is necessarily in a difficulty; he can only study "average cow" which is a compound containing parts not only of sick cow but also underfed cow and mismanaged cow, for which reason the writer, enjoying facilities for applying tests to large numbers of animals whose every surrounding circumstance is known to him, prefers to rely on a study of their individual life histories.

A selection of records of cows subjected to artificial control is exhibited in Table III. They speak for themselves. In that of the cow Edna the average of her 1st lactation was only 14·8; through unknown cause she failed to conceive till 196 days after that calving, whereupon in the 2nd lactation her daily yield doubled. The result of the early service (44 days) during the 2nd lactation is that the 3rd lactation does not show normal progress. The daily average actually falls. Yet again preceding the 4th lactation a short service period was allowed (53 days) and though the total yield is large it occupied 374 days and the daily average shows yet another fall compared with 2nd lactation. Then she is put under control and a long interval brings out what she is really capable of. The total rises from about 10,000 to 15,000 lb., the daily average from 30 to 42·5.

Cow Disk teaches the same lesson. The rise and fall in daily averages with long and short calving intervals *preceding* is striking. Cow Carnation controlled from 1st calving shows an orderly progression in her yields up to the 3rd calving but then, on the C.I.P. being shortened, there is no

Table III.

Calving interval preceding	Days dry included	Yield	Daily average in lactation	Length of S.P. during lactation	Daily average over days dry preceding and days in milk
		EDNA. *Half Friesian*			
		Service unrestricted			
(1st lactation)	—	6,521	14·8	196	—
476	38	6,750	30·0	44	25·6
324	99	7,031	28·6	53	20·4
333	88	10,345	27·6	222	22·3
		Service prevented for 63 days			
502	128	15,324	42·5	176	31·4
456	96	12,938	37·7	91	29·4
371	28	13,663	29·9	304	28·2
		DISK. *Half Friesian*			
		Service unrestricted			
(1st lactation)	—	5,591	20·5	46	—
326	54	6,074	17·6	98	15·2
378	33	5,438	19·7	114	17·2
394	119	9,946	31·2	65	22·7
345	27	6,386	24·0	45	21·7
325	59	8,159	23·3	148	19·9
		Service prevented for 63 days			
428	78	10,417	34·1	68	27·7
350	51	10,534	31·8	118	27·4
398	65	10,863	31·8	115	26·7
395	54	10,575	33·0	111	28·0
		CARNATION. *Three-fourths Friesian*			
		Service restricted for varying periods from 1st calving			
(1st lactation)	—	10,052	22·7	216	—
496	55	11,129	34·0	111	29·1
391	64	11.838	39·4	52	32·5
		Service unrestricted			
332	32	11,756	35·0	120	31·9

further progress but a slight fall in the daily average. That lactation is specially interesting in that the short interval was in breach of orders and when the Manager was reproached he undertook to make good any adverse effect by special feeding in the two months before next calving. He is an expert feeder but failed as is seen.

These are, of course, selected cases and some could have been found which if they did not suggest contrary conclusions would give little support to those adopted, but any such, where there are no other factors known to be operating, are few indeed.

For comparison Table IV is made up from histories of cows never controlled.

The recuperative value of long intervals is equally clear but long intervals occurring through defects and breakdowns bring with them

excessively long D.P.'s. In this table reference is invited particularly to cow Poppy, belonging to the herd which supplies Table I, which shows a progressively shortening interval and a progressively falling yield from 1st calving. Yet she reproduces freely and no evidence is forthcoming of any deleterious factor except short C.I.P.

Table IV. *Intervals varied by environment only.*

All Ayrshire crosses except Poppy.

Days dry preceding	Service period in preceding lactation	Yield	Average daily while in milk	Days dry preceding	Service period in preceding lactation	Yield	Average daily while in milk
	Fidra				Poppy		
First	—	4,969	14·0	First	—	2088	9·9
14	83	4,551	14·8	135	65	3803	13·4
34	60	4,557	15·2	40	44	3381	11·7
117	137	5,454	19·0	34	44	2465	11·6
39	45	2,747½	10·3	94	26	2704	11·5
159	145	8,140	21·1				
	Acorn				Certainty		
First	—	7,315	17·7	First	—	7409	25·9
—	133	3,495	11·6	30	36	4919	20·8
16	36	9,248	19·5	88	44	5606	19·4
106	299	10,085	35·5	31	40	7534	23·1
34	38	7,252	23·8	279	325	9395	27·5
107	132	11,101	34·0	54	115	7589	26·6
19	66	5,674	19·3				
42	61	4,229	12·2				
	Salsette				Louise		
First	—	9,648	25·5	First	—	2963	8·9
18	116	5,933	20·0	25	76	2181	9·5
87	104	7,304	19·4	89	37	1952	10·2
60	156	9,934	25·0	108	20	3272	11·5
53	172	8,908	20·2	22	25	2887	15·6
37	199	10,543	26·0	118	23	4144	13·1
				153	188	6454	26·2

Absence of relation between S.P. *and yield in the accompanying lactation.*

Table III (and also Table IV) throws considerable light on this point. The relation of daily yield with C.I.P. is clear but with current S.P., which the fifth column is included to show, it cannot be identified.

There is some indication that moderate shortening of S.P. will *raise* current daily yield which is more or less to be expected, as pregnancy will be advanced thereby, the D.P. shortened and drying off occur while the yield is still high. But if so it is paid for in the next lactation.

Relation of C.I.P. *to age.*

The matter is really one of mammary maturity of which neither age nor size is in itself a criterion. The writer has found it useful to work

on an assumption that the smaller the daily yield of a 1st calf heifer *relative to the average of her class* the longer should be the C.I.P. for the 2nd lactation; in which connection Table V has been prepared. It contains histories of all the cows of a certain class (Hybrids) in two herds, which completed their first two lactations between certain dates. These herds had first observed a general restriction of service to the 63rd day, then all restrictions were removed for about a year, followed by reimposition with directions which resulted in giving 120 day intervals to all extra low and extra high yielders. In all there are included young cows under no restriction, others restricted for 63 days and those restricted 120 days.

Table V. *Young Hybrid cows—long and short intervals.*

Cow	Age 1st calving years	months	Daily average yield 1st lact.	C.I.P. 2nd lact.	D.P. before 2nd lact.	Daily average 2nd lact.	% increase daily average
			(*a*) Service prevented till 120 days after calving				
B. Opal	2	4	17·0	448	116	25·7	51·1
Tulip	3	6	18·2	419	79	28·7	57·6
Southi	2	6	16·9	422	77	28·0	65·6
Harford	2	8	15·3	406	77	24·8	62·0
Chinchala	2	9	11·2	516	122	17·1	52·6
Kenford	2	8	16·6	424	95	22·5	35·5
Piyaria	3	2	12·4	430	85	21·8	75·9
Red Rose	2	5	12·3	489	113	17·4	41·4
C. Pretty	2	11	44·1*	424	82	49·0*	11·0
Parbatti	3	0	29·4	472	74	42·0	42·8
Mean	2	9½	19·3	445	92	27·7	49·5
			(*b*) 1st five restricted till 63rd day; 2nd five unrestricted				
Bhatori	3	0	27·8	382	76	39·6	42·4
Hainford	2	5	13·1	389	83	15·7	19·8
C. Primrose	3	0	16·0	344	62	22·0	37·5
C. Parrot	2	10	12·4	384	128	21·7	74·5
Cotton Tail	3	1	16·5	367	69	22·0	33·3
Beauty	1	10	16·5	331	46	23·4	41·8
Olive	3	1	22·3	326	100	23·9	7·1
Jasford	2	6	15·2	328	60	19·3	26·9
C. Polly	2	10	13·8	333	33	12·7	−7·9
B. Mango	3	0	19·6	342	54	16·9	−13·7
Mean	2	9	17·3	352	71	21·7	26·1

* Average of 300 days after calving as 2nd lactation incomplete.

As is to be expected one or two do not follow the rule of the majority but the majority seem definitely significant.

Late mammary development is a hereditary feature of many strains of the Indian breeds to which the female parents of these animals belonged.

It is of some interest to note further that of the three extra high yield cows in the middle of the table, Nos. 9, 10 and 11, the earliest

occurrence of oestrum was on the 84th day after calving—*i.e.* not until the daily yield had fallen appreciably from its highest levels.

Relation to longevity.

Reference is invited to Table IV. In India the standard of milking capacity is low, yet even there any decent herd should have got rid of cow Louise long before a chance long interval showed her to be well above the local average quality. So, it is suggested, many farmers' cows in Europe are purchased and discarded again as disappointing, when the sole, and unknown, reason of failure was the short C.I.P. in the hands of the previous owner.

Again, many 2nd calvers are found not to progress satisfactorily in yield over their 1st lactations, for want of an adequate interval, and are condemned. Many good cows are slow in maturing, although they appear well grown, and require exceptionally long intervals even if they calve for the first time at as much as 3 years of age. If these points are thought over it is not unreasonable to visualise a steady stream of potentially good cows going to the butcher, and in this aspect of the matter the question of whether the influential factor is the calving interval preceding or current is of more than academic interest.

DISCUSSION.

It is desirable to refer here to the fact pointed out by Gaines (6) that every lactation has a high level occurring soon after calving and to his deduction that the more often it recurs—involving greatest possible frequency of conception—the higher must be the yield in life. Superficially that presents a formidable difficulty to acceptance of the contentions here put forward. If in fact the influential factor is the length of the current interval it is irremovable. The optimum service time will then be very short (a separate study of the current effect of S.P. puts it immediately after the peak of lactation, say at the 40th day) and both Sanders' conclusions and the writer's appear to be demolished. But if the strong influence of the preceding interval is admitted the whole matter is easily explained. Without adequate preceding interval the high level in the new lactation, which is indispensable to the view favouring frequent pregnancy, is not reached. The writer has plotted the curves of the lactations in Table V. The steep rise to high levels in those that follow long intervals and their difference from the others, in this respect, is remarkable.

The problem is essentially one of nutrition. From concurrent studies

it would appear that the average cow is never able to maintain her nitrogen and mineral balances, particularly the latter, during the high levels of lactation. But in the progressive fall of the milk yield, at a point somewhere between daily yields of two gallons and one gallon, equilibrium is established and storage follows. Provided the diet is complete, the maximum daily yield in the following lactation will then vary with the time between the beginning of "Storage" and the next calving.

It is seen here that "Dry Period" is merely a part of the recuperative phase and is not a factor whose influence can be measured by itself.

Summary.

Herd statistics of cattle in India suggest that for animals of about 3500 lb. yield capacity a calving interval of rather less than a year will give the best results in the following lactation. For cattle whose yield capacity is 6000 lb. an interval of 420 days is desirable while one of less than 335 days is seriously injurious.

Study of individual cows and their recorded histories reinforces the above conclusions and also suggests that the interval should be longer in early lactations than in late and progressively longer as the milking capacity increases.

It also indicates that unrestricted access to the bull may prevent the real yield capacity of a cow being discovered.

A relation between daily or weekly yield and the length of calving interval during the same lactation is not found.

It is now suggested that "Calving Interval" should be controlled by the following law:

"The optimum calving interval varies directly with milking capacity and inversely with age up to maturity."

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THE EFFECT OF FRESH STRAW ON THE GROWTH OF CERTAIN LEGUMES.

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(With Two Text-figures.)

In field trials made at Rothamsted during 1917–1918 (1) to test the comparative value of farmyard manure and of artificials as fertilisers for clover, the former gave remarkably better results. Its effect not only surpassed that produced by any mixture of artificials tested, in the year of application, but was evident even when applied three years before the clover was sown. In comparing the action of farmyard manure and of artificials, the effect of the former on the physical condition of such a heavy soil as that at Rothamsted must always be considered. In these trials, however, the superiority of farmyard manure was so much more marked with clover than with cereal and root crops on the same soil, that some further explanation seems to be required. Since clover is a legume, manures may have both a direct action on the plant, and may also affect its growth indirectly, by influencing the formation or activity of the nodules upon its roots. The purpose of the work here described was to determine whether farmyard manure stimulated nodule development on legumes and, if so, to what constituent of the manure this stimulation was due.

In order to separate the direct effect on the plant from any indirect influence through the nodules, farmyard manure and its constituents were first tested both on nodule free and on inoculated plants. Preliminary trials showed that, owing to the widespread occurrence of nodule organisms in soil, none of the common British legume crop plants could be grown without nodules, unless an unnatural medium such as sterilised soil or sand were employed. It was found, however, that the soy bean (*Glycine hispida*) does not produce nodules in Rothamsted soil unless infected with a culture of the appropriate variety of the nodule bacteria. This plant was therefore used in the first two experiments here described.

In the first experiment, pots of soil and sand mixture were prepared

and manured as shown in Table I[1]. There were ten pots of each treatment, five sown with seed sterilised by the method of Hutchinson and Miller (2) and five with sterilised seed inoculated with a suspension of the soy bean nodule organism. Three seedlings were allowed to grow in each pot and, after 13 weeks' growth in a glasshouse, the dry weights of tops and roots were obtained and the nodules counted, the soil being washed from the roots with a fine stream of water from a hose. The results of the experiments are shown in Table I.

Table I. *Pot experiment with soy beans.*

Series	Manuring per pot	Dry weight tops in gm. Means of 5 pots and standard errors	Dry weight roots (gm.)	Total nodules and standard errors	Nodules per gm. of root
	Not inoculated:				
1	1 lb. farmyard manure	4·6 ±0·92	1·67	—	—
2	½ lb. farmyard manure	5·4 ±0·81	2·02	—	—
3	Extract from ½ lb. farmyard manure	8·1 ±0·66	2·87	—	—
4	No manure	2·5 ±0·17	1·95	—	—
5	½ lb. fresh chaff	1·5 ±0·12	1·98	—	—
6	½ lb. rotted straw	3·0 ±0·17	1·27	—	—
	Inoculated:				
7	1 lb. farmyard manure	7·6 ±0·63	1·51	146·2 ±4·2	96·8
8	½ lb. farmyard manure	6·24 ±0·61	1·75	153·2 ±3·3	87·5
9	Extract from ½ lb. farmyard manure	8·56 ±0·84	2·18	94·0 ±2·5	43·2
10	No manure	2·3 ±0·24	1·41	63·2 ±3·0	44·8
11	½ lb. fresh chaff	2·0 ±0·18	1·11	105·0 ±4·0	94·6
12	½ lb. rotted straw	2·9 ±0·41	1·28	65·0 ±3·2	50·8

The manure and its extract have produced a considerable increase in the growth of the tops. This is mainly due to a direct action on the plant since it occurs in the absence of nodules. In the uninoculated series fresh straw has reduced the growth of the top as compared with the unmanured set and the farmyard manure which contained a lot of unrotted straw has produced less increase than the manure extract. The presence of nodules has not produced any significant increase in yield save in these same three sets (7, 8 and 11) that contained fresh straw. The yield of sets 7 and 8 containing farmyard manure are not significantly lower than that of set 9 containing manure extract, while there is no significant difference

[1] Each pot contained 23 lb. of a mixture of two parts soil and one part sand. The manure extract was prepared by soaking manure in water at the rate of 3 litres to 8 oz. Each plant was given the extract from 8 oz. manure during its growth period. The manure contained 0·92 per cent. nitrogen while the extract contained 0·43 per cent. nitrogen. Series 6 and 12 were given straw rotted by the "Adco" process.

in yield between the set containing chaff (11) and the unmanured[1]. The inoculation has thus prevented the straw from lowering the yield. This suggests that its depressing action in the uninoculated set was the result of nitrogen starvation due to the well-known effect of straw in rendering nitrates unavailable by encouraging their assimilation by micro-organisms. Where the nodule organism rendered the plant independent of external nitrogen compounds, this harmful action did not occur. In the inoculated sets, the manurial treatment has greatly affected the number of nodules produced. When allowance is made for unequal root development by taking the nodule numbers per gram of root, the infection has been increased only by chaff and by the manure containing straw. In the case of sets 7, 8 and 9, however, greater root development induced by manure and its extract caused the appearance of more nodules owing to the greater surface exposed to infection. The following experiment was made to see whether the increased nodule formation induced by unrotted straw could not be similarly enhanced by the

Table II. *Pot experiment with soy beans.*

Set	Manuring per pot	Dry weights of top and standard errors (gm.)	Dry weights of roots and standard errors (gm.)	Nitrogen in tops %	Nitrogen in tops Total content (gm.)	Nodule nos. and standard errors	Nodules per gm. root
	Not inoculated:						
1	No manure	21·2 ± 2·5	—	1·56	0·33	—	—
2	4 oz. chaff	19·1 ± 1·4	—	1·99	0·38	—	—
3	3·3 gm. K_2HPO_4	28·8 ± 2·2	—	1·42	0·41	—	—
4	3·3 gm. K_2HPO_4 + 4 oz. chaff	24·6 ± 2·2	—	1·37	0·34	—	—
	Inoculated:						
5	No manure	23·1 ± 2·6	3·1 ± 0·3	3·16	0·73	121·8 ± 14·0	39·3
6	4 oz. chaff	26·7 ± 2·5	4·5 ± 0·5	3·61	0·96	179·8 ± 9·9	39·9
7	3·3 gm. K_2HPO_4	31·8 ± 3·5	6·4 ± 0·2	2·41	0·77	106·4 ± 4·8	16·6
8	3·3 gm. K_2HPO_4 + 4 oz. chaff	33·0 ± 1·7	6·9 ± 0·13	2·96	0·98	316·8 ± 41·7	45·9

addition of soluble potassium phosphate to replace the potash and phosphate supplied in the manure. Soy beans were grown in pots, containing soil and sand mixture, which were given the manuring shown in Table II. The salts in sets 3, 4, 7 and 8 were applied as solutions during the growth

[1] In testing the significance of these differences, the t test was used (see Fisher (3)). In comparing the mean yield of tops in sets 8 and 9, $t=2·2$, giving a value of P between 0·1 and 0·05. In a similar comparison between sets 10 and 11, $t=1·05$, the value of P lying between 0·4 and 0·3.

of the plants. Ten pots of each treatment were prepared, five sown with inoculated and five with sterilised seed. After 12 weeks' growth in a glass-house, the nodules were counted, dry weights taken and nitrogen estimations made of the tops. The results are shown in Table II.

The root development has been increased in the pots given potassium phosphate and in set 8 the further addition of chaff caused a large increase in nodule numbers. The presence of nodules has increased the nitrogen content in all inoculated sets but has caused a significant increase in the top growth only in sets 6 and 8 where chaff was present[1]. The chaff has enabled the plant to make some use of the nitrogen fixed by the bacteria, to increase its growth. The action of chaff in increasing nodules can be explained as being due to its contained carbohydrate material enabling the bacteria to multiply in the soil. It seems probable that the pentosans and starches in the straw, being readily attacked by bacteria, are the principal sources of energy. This view is consistent with the finding in Experiment 1 that rotted straw did not appreciably increase the nodule numbers. Fresh straw should therefore produce greater effect in stimulating nodule formation than straw that has been mixed in the soil some time and has lost its more easily decomposed constituents before the bacteria are added. The following experiment was made to test this

Table III. *Pot experiment with* Vicia faba *L.*

Set	Manurial treatment per pot	Dry weight of tops and standard errors (gm.)	Nodule numbers and standard errors
	Series *A*. Straw and phosphate added at time of sowing:		
1	Unmanured	$19{\cdot}6 \pm 1{\cdot}12$	$234{\cdot}8 \pm 19{\cdot}6$
2	3 gm. $CaH_4(PO_4)_2 + 2H_2O$	$25{\cdot}3 \pm 1{\cdot}02$	$302{\cdot}0 \pm 36{\cdot}6$
3	8 oz. chaff	$22{\cdot}4 \pm 2{\cdot}05$	$636{\cdot}5 \pm 45{\cdot}4$
4	3 gm. $CaH_4(PO_4)_2 + 2H_2O$ + 8 oz. chaff	$33{\cdot}7 \pm 1{\cdot}28$	$866{\cdot}2 \pm 47{\cdot}1$
	Series *B*. Straw and phosphate added one month before sowing:		
5	Unmanured	$17{\cdot}9 \pm 0{\cdot}96$	$254{\cdot}5 \pm 16{\cdot}0$
6	3 gm. $CaH_4(PO_4)_2 + 2H_2O$	$18{\cdot}0 \pm 1{\cdot}62$	$267{\cdot}8 \pm 20{\cdot}8$
7	8 oz. chaff	$17{\cdot}3 \pm 1{\cdot}97$	$494{\cdot}5 \pm 54{\cdot}9$
8	3 gm. $CaH_4(PO_4)_2 + 2H_2O$ + 8 oz. chaff	$24{\cdot}5 \pm 2{\cdot}83$	$725{\cdot}0 \pm 22{\cdot}9$

point and to confirm the results of Experiment 2 with a different plant. Pots containing 22 lb. of soil-sand mixture were given the manurial treatments shown in Table III. Two series, each having five parallel pots of each treatment, were run; in one series the manurial dressings were

[1] In comparing the mean yields of top in sets 3 and 7, $t = 0{\cdot}73$, giving a value of P lying between 0·5 and 0·4, whereas between sets 2 and 6, $t = 2{\cdot}9$, $P = 0{\cdot}02$, and between sets 4 and 8, $t = 2{\cdot}97$, $P = 0{\cdot}02$.

added at the time of sowing and inoculation, while, in the other, they were added one month before this, the soil being kept moist. Three broad beans (*Vicia faba* L.) were grown in each pot and inoculated at the time of sowing with a thick suspension of the *Vicia* nodule organism. The results, after three months' growth in a glasshouse, are shown in Table III.

The chaff produced a large increase in nodules, the numbers of which, as in the last experiment, were greater where phosphate is also present. The straw had more influence on nodule numbers when applied fresh at the time of sowing and inoculation than when applied one month before. The freshly applied chaff and phosphate greatly increased the yield when combined, the phosphate alone produced a smaller, but significant increase and the chaff alone an increase whose significance is doubtful. When added one month before sowing and inoculation, the chaff and phosphate significantly increased the yield only where both were present, and then to a smaller extent than when added fresh at the time of sowing[1]. This experiment thus indicates that the more quickly decomposed fractions of the straw are effective in increasing nodule numbers and yield.

Table IV. *Plot experiment on Little Hoos Field, Rothamsted.*

		Yield off 1/100 acre plots in lb.			
		1923. Beans, pods and straw		1924. Wheat, grain and straw	
Plot	Treatment in quantities per acre		Means		Means
1	No manure	28		21	
6		29·5	28·75	24·5	24·88
11		30		23·5	
16		27·5		30·5	
2	5 tons chaff	33·5		30·5	
7		29	33·33	24	29·17
12		37·5		33	
3	400 lb. superphosphate	27		20	
8		29	27·83	28	24·83
13		27·5		26·5	
4	5 tons chaff + 400 lb.	39		33·5	
9	superphosphate	31·5	34·66	23	30·33
14		33·5		34·5	
5	400 lb. superphosphate +	36		23	
10	200 lb. sulphate of	41·5	36·5	36	29·33
15	potash	32		29	

The pot experiments described above indicate that the harmful effects of incorporating fresh straw into the soil do not show on a succeeding

[1] In comparing the means of sets 1 and 2, $t=3·75$, giving P a value of 0·01. In comparing sets 1 and 3, $t=1·44$, the value of P lying between 0·2 and 0·1. For sets 5 and 8, $t=2·54$, P having a value between 0·05 and 0·02.

crop of beans or soy beans that is supplied with necessary nodule organisms. It seems possible that this principle might be made use of under field conditions to enable fresh straw to be ploughed in without harmful results. In order to test the action of straw manuring under field conditions, the following experiment was made on Little Hoos Field. The treatments shown in Table IV were tested in 1/100 acre plots. Three plots of each treatment and four unmanured plots were laid down in a randomised arrangement. Beans were sown in April 1923 and reaped in September, and the following season wheat was grown on the same ground. The yield of the two crops is shown in Table IV. The superphosphate alone has not increased the crop, chaff has appreciably increased both the bean crop and the succeeding wheat crop. This effect can also be produced by means of superphosphate + sulphate of potash.

Discussion.

The ploughing in of unrotted straw produces physical and chemical changes in the soil and also influences the micro-organic population. It has a marked effect in lightening a heavy soil and improving its aeration. There is some evidence that fresh straw contains or produces substances that may be directly toxic to plants but that this factor is of no importance in clay soil (4). The most important chemical changes produced by fresh straw result from the fact that it supplies readily available carbon compounds which are utilised by the soil micro-organisms, so that these multiply rapidly and assimilate the soil nitrates in competition with the crop plants. These effects have been studied by numerous workers (see Murray (5) and Martin (6)). That a similar loss of nitrates takes place under the conditions of pot culture obtaining in the experiments described is shown by the following observations. Two sets of duplicate pots similar to those used in Experiment 1 were filled, the first set with soil-sand mixture only, the second with this mixture plus 8 oz. of chaff. The pots were kept in a greenhouse and maintained at a water content of 14 per cent. and, after one, two and three months, the nitrate, water soluble P_2O_5 and water soluble K_2O were determined. The results are shown in Fig. 1. The straw caused a considerable loss of nitrates, while the soluble potash was increased, in the first three months after its incorporation with the soil.

Where the succeeding crop is a legume, supplied with its nitrogen-fixing bacteria, it was reasonable to suppose that no loss of yield would result from the depletion of the soil nitrates produced by the straw. The

experiments described above show that in the case of inoculated soy beans and broad beans there was in fact no loss of yield from this cause, and that with the latter, where additional phosphate was also supplied,

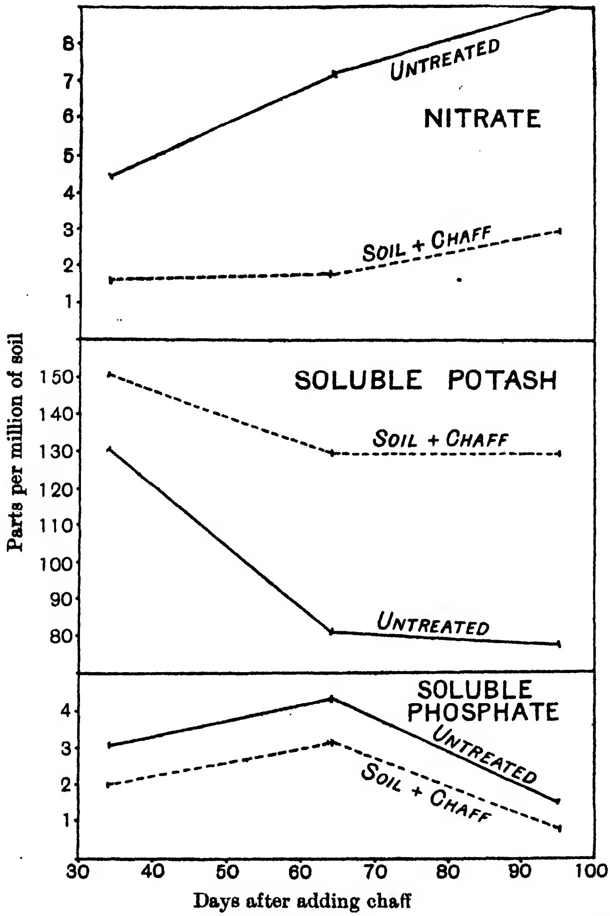

Fig. 1. Effect of chaff on the nitrate and on the water soluble potash and phosphate in pot soil.

there was an actual increase in yield due to the straw. This increase was associated with the development of a considerably greater number of nodules in the presence of the straw.

The increase in nodule numbers by the straw would seem to be explained by the fact that the straw can be utilised as a source of food supply by the nodule organism in the soil. (See Appendix.)

The initially harmful effect of ploughing in fresh straw has deterred its use, although the benefits eventually resulting from the increase in soil organic matter are recognised. By the use of a bean crop, following its application, this harmful action may perhaps be avoided and the crop actually benefited by the straw. It seems possible that further experiments along this line on different soils and with various legumes may enable the humus content of heavy soils to be raised by straw manuring without temporary loss in crop.

APPENDIX.

THE INFLUENCE OF FRESH CHAFF AND OF CALCIUM PHOSPHATE ON THE MULTIPLICATION OF THE NODULE ORGANISM IN SOIL.

By P. H. H. GRAY.

In order to test the effect of chaff and of phosphate upon the multiplication of the nodule organism in soil, the following experiment was carried out.

One hundred grams of moist sieved soil from the unmanured plot on Hoos Field were placed in each of four Erlenmeyer flasks and the following substances were added.

Flask *A*: 2 gm. of chopped oat straw.

Flask *B*: 0·025 gm. of $CaH_4(PO_4)_2 + 2H_2O$.

Flask *C*: 2 gm. of chopped oat straw + 0·025 gm. $CaH_4(PO_4)_2 + 2H_2O$.

Flask *D*: No addition.

The flasks were plugged with cotton wool, sterilised in the autoclave at 15 lb. pressure for half an hour and inoculated with a pure culture of the lucerne nodule organism, six days old, by adding to each flask four drops from a suspension of the culture in physiological salt solution. Bacterial numbers were estimated at intervals by making plate counts of the soil on an agar medium containing mineral salts, sucrose and extract of lucerne roots. The growth of the organisms is shown in Fig. 2, where each point represents the mean count from four parallel plates.

The chaff has greatly increased the bacterial numbers whether added alone or with phosphate. The phosphate has stimulated multiplication at the commencement, especially where added together with chaff.

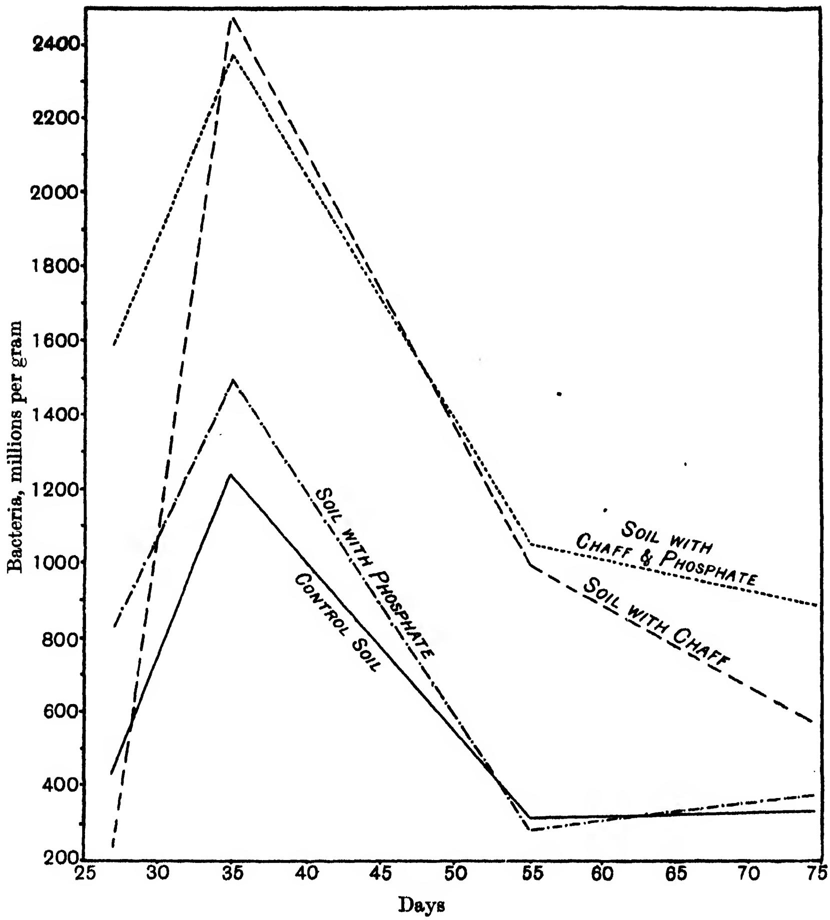

Fig. 2. Growth of the lucerne nodule organism in sterilised soil with and without chaff and $CaH_4(PO_4)_2 + 2H_2O$.

SUMMARY AND ABSTRACT.

1. In pot experiments with *Glycine hispida* and *Vicia faba* L., fresh chaff incorporated with the soil caused a significant increase in the number of nodules produced on inoculated plants, this increase being augmented by the further addition of phosphates.

2. Fresh chaff, added at the time of sowing and inoculation, had more effect than chaff which was allowed to decompose in the soil for a month.

3. Fresh chaff increases the multiplication of the nodule organism in sterilised soil.

4. In soy beans without nodules, the chaff depressed the growth of the tops, but this depression did not occur either with soy or broad beans where nodules were present.

5. In a field experiment made at Rothamsted, chaff, freshly ploughed in, increased the growth of broad beans and also of wheat sown the next season on the same ground.

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A WIDESPREAD OCCURRENCE OF XANTHIN CALCULI IN SHEEP.

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(With Plates II and III.)

Introduction.

Xanthin (2·6 dioxypurin) has been recognised for more than a century (1) as a rare constituent of urinary calculi. It is present in human urine (2, 3) and the amount is increased during inflammation of the kidneys (4). Its presence in the urine of sheep (5) and of pigs (6) was recognised at an early date. It occurs in blood, the muscles and many of the glands of animals and is widely distributed in the vegetable kingdom. This common occurrence is not surprising since xanthin is a regular though secondary product of the hydrolysis of nuclein (7, 8). Owing probably to the action of xanthin oxidase in the liver and other organs, the quantity of xanthin in urine and blood, under normal conditions, is very small and concretions of this compound are regarded as almost the rarest of urinary calculi.

During the course of an investigation at the Cawthron Institute into the mineral contents of Nelson pastures, the occurrence of stone in the kidneys of sheep was pointed out by several farmers in different parts of the Waimea County. Further inquiry and the careful examination of a number of kidneys showed

(1) that on some farms kidney calculi were very prevalent;

(2) that in every case the calculi consisted of xanthin;

(3) that serious loss of stock from calculus trouble was restricted to farms on one particular soil type which was of low fertility.

These facts suggest that this abundant occurrence of xanthin calculi may be caused by deficiencies in the pastures associated with the soil type in question. The analyses in connection with a comprehensive soil survey of the Nelson district by the agricultural division of the Cawthron Institute had already shown that the soil is very deficient in lime and phosphoric acid. The presence of calculi in the kidneys of cattle fed on a phosphate deficient diet has been noted in South Africa (9); the chemical nature of these calculi was not however stated.

The occurrence of xanthin calculi amongst stock pastured upon one particular type of soil suggests many questions of physiological interest.

(1) Can a deficiency of certain elements in the soil increase the formation of xanthin or its precursors in the fodder?

(2) Can an unbalanced mineral ration cause a partial or complete arrest of purin metabolism at the xanthin stage by reducing the secretion or activity of xanthin oxidase?

(3) Can the mineral ration affect the purin metabolism at other stages and thus have a bearing on the guanin deposits in pigs and uric acid calculi in other animals?

(4) Is xanthin accumulation and deposition merely determined by some simple cause such as a change in the pH value of the blood or urine?

It is proposed to initiate feeding experiments on the formation of xanthin calculi.

Occurrence of the calculi.

All the calculi which have come to the notice of the authors have been taken from stock raised on soil of the Moutere Hills type. This soil covers an area of 300–400 sq. miles stretching as a belt nearly 50 miles in a north to south direction from Motueka in Tasman Bay to Top House at the foot of St Arnaud's Range. Cases have been seen or reported from eight districts, but the occurrence has been most striking in Pigeon Valley near Wakefield.

In this valley, on one small farm grazing 120 sheep, 10 sheep have been found in the last six months with kidney calculi as a predisposing cause of death. The owner can pick out the badly affected sheep by their appearance and killed two sheep in the presence of the authors to demonstrate this. In both cases the kidneys were badly affected.

No instance of calculus trouble has been reported in the case of sheep reared on the more fertile soils of the Waimea County. On the few areas of Moutere Hills soil where, by lime and phosphate treatment, good pastures have been established the presence of calculi in the kidneys of sheep has likewise not been recorded.

Though the calculi generally consist of a gravel made up of a mixture of large and small stones with particles as fine as dust (Fig. 1) a calculus from Harakeke represents a complete cast of the enlarged kidney pelvis. The specimen weighs 36 gm. (Fig. 2). Small stones are frequently found in hoggets which are in sufficiently good condition to warrant their slaughter for meat.

Large stones may occur in both hoggets and full-mouthed sheep.

Wethers and ewes seem equally affected. Several farmers have stated that they have found similar stones in the kidneys of cattle and hares on Moutere Hills country but none of these calculi has come under the immediate notice of the authors.

IDENTIFICATION OF THE XANTHIN.

Though the calculi from the sheep on the Moutere soil are of a light grey stony appearance suggestive of calcium carbonate or phosphate they have proved in every case to be composed of xanthin, $C_5H_4N_4O_2$. This is remarkable since xanthin calculi are regarded as extremely rare.

In a particular case the dried calculus left only 0·1 per cent. of ash on ignition. It contained 35·35 per cent. of nitrogen, which is surprisingly near to the value 36·8 per cent. required for pure xanthin. After purification by solution in ammonia, decolorisation with animal charcoal and reprecipitation by spontaneous evaporation of the ammonia, the xanthin was obtained in characteristic microscopic globules containing 36·6 per cent. of nitrogen, thus agreeing well with the calculated value.

The xanthin showed the following qualitative reactions which confirm its identity:

(1) It dissolved in ammonia and more readily in caustic soda and was reprecipitated by dilute acetic acid.

(2) Evaporated to dryness with nitric acid it left a yellow residue which gave a yellow solution with ammonia and deeper yellow with caustic soda. The soda solution became purple-violet when gently warmed.

(3) Evaporated to dryness with hydrochloric acid and chlorate of potash it left a residue which turned violet with ammonia.

(4) The solution in baryta water upon treatment with diazotised sulphanilic acid yielded a brick-red precipitate (10).

SYMPTOMS SHOWN BY THE AFFECTED SHEEP.

Affected sheep are frequently in poor condition. In a number of cases there was no fat surrounding the kidneys (Fig. 3). Two 2-toothed sheep with large calculi in the kidneys had a live weight of 49 lb. and 52 lb. respectively. Healthy sheep of the same age should weigh not less than 80 lb. In severe cases the sheep mope around the paddocks and show little inclination to graze. Frequently such sheep have arched backs with the hind-legs drawn under the body (Fig. 4). There appears to be great difficulty in urination and the sheep may be seen straining

frequently. One sheep badly affected was distinctly anaemic in appearance.

In severe cases one or both kidneys may be greatly enlarged and full of calculi.

Sometimes rupture of the kidney takes place and liquid accumulates in the body cavity, quickly causing death.

Two typical cases of the occurrence of calculi in the kidneys of sheep are set out below.

Sheep I. Hogget (12 months). Bred and died on the property. One enlarged kidney containing a number of small stones ranging up to $\frac{3}{8}''$ in diameter. The other kidney was normal in size but punctured. The bladder had apparently stopped functioning. About 2 gallons of turbid liquid were found in the body cavity.

Sheep II. Ewe (4 years). Bred and died on the property. Sheep in fair condition. Both kidneys contained stones and powder.

One kidney very much enlarged. Some of the calculi were $\frac{1}{2}''$ in diameter in addition to numerous small stones and powder. The calculi from the large kidney weighed 41·71 gm., those from the smaller kidney 15·41 gm.

Stock conditions on the Moutere Hills country.

On many parts of the soil of the Moutere Hills type the results obtained by farmers from stock—both sheep and cattle—compare unfavourably with those obtained in other parts of the Nelson district where the soils possess higher fertility.

The poorer results associated with stock on Moutere Hills country are of the following nature:

(1) Low lambing returns. A census of the percentage of lambs on 33 farms in poorer parts of the Moutere Hills country shows that the average flock increase was 40 per cent. in the 1927 season. In certain instances the percentage of lambs tailed has fallen as low as 20 per cent.

(2) Mortality among sheep and cattle. On certain farms serious losses of stock have occurred. Some farmers state that the increase to the flock by lambs just balances the losses caused by the death of hoggets and ewes. In some seasons the loss of hoggets is particularly high.

(3) Inability to fatten lambs or hoggets on any pastures on the Moutere type of soil unless lime and phosphate treatment has been given to the land or unless supplementary feeding is undertaken.

(4) Comparatively low wool returns. In extreme cases $3\frac{1}{2}$ lb. of wool per sheep is all that is obtained.

(5) Undersized stock are a general feature—more pronounced in the case of cattle than sheep (Fig. 5). Calves are difficult to rear and are slow to mature.

(6) The bones of both ewes and cows are frequently very light and are easily fractured.

(7) In many cases both sheep and cattle on Moutere Hills pastures are in poor condition. Cows give very little milk. Meat is of poor quality.

(8) Many instances have been reported where stock have eaten earth and chewed bones and sticks.

(9) Mixtures of bonemeal and salt are eaten greedily by both cattle and sheep on several farms where a poor condition of stock is noticeable. Great improvement in the condition of the stock has resulted from bonemeal feeding.

EXTRACT FROM THE REPORT OF MR C. S. HOPKIRK, B.V.Sc. (DEPT. OF AGRIC.), ON CERTAIN ORGANS OF A SHEEP AFFECTED WITH XANTHIN CALCULI.

Mr Hopkirk, officer in charge of the Wallaceville Veterinary Laboratory, has kindly supplied a report on certain organs of sheep affected with calculi, from which the following extract is quoted:

"*Heart Muscle*—normal.

Bladder—normal.

Thyroid Gland—showed some abnormal activity. The follicles contained much colloid which, owing to shrinkage, incompletely filled the cavities. Lining cells were mainly cuboidal, and apparently in some of the acini there had been some degree of sloughing of cells, and in others a definite proliferation.

Liver—showed marked degeneration of many individual cells, and a vacuolated condition of the remainder. Some of the sinusoids contained xanthin-like granules.

Kidney.—This organ, as one would expect, showed the greatest change. The glomerular tufts were shrunken and cells pyknotic. Glomerular sacs contained a certain amount of albumen. The proximal convoluted tubules were markedly eosinophilic, and necrotic, the lining cells having sloughed in many of the tubules while others contained swollen, vacuolated, non-nucleated cells. Some deposit was noticeable in the lumen of the tubules but this debris was to be seen in greater amount in the distal convoluted tubules, and even in the collecting vessels where it had the yellow appearance of the xanthin calculi. The medulla of the kidney showed definite pressure atrophy with cirrhosis, while there was a further pressure atrophy of the subcapsular portion of the cortex."

The Moutere Hills soil.

This soil type covers some 300–400 square miles of country in the Waimea County. It appears to have resulted from the weathering of large alluvial deposits laid down by some ancient river of Pleistocene age. These deposits consist of well-weathered gravels mainly composed of sandstone, claystone and greywacke, mixed with sand and fine silt. The topsoil is a loam containing some 11 per cent. of "clay," while the subsoil may best be classed as a sandy clay.

The soil has excellent textural qualities and has proved ideal for apple culture when suitably manured. Exotic trees such as *Pinus radiata* make wonderful growth on even the poorest areas of this soil.

The chemical analysis of Moutere Hills soil compared with analyses of "Bush" sick and "Waihi" disease soils is shown in the following table:

Table I. *Chemical analysis of Moutere Hills soil.*

		Moutere Hills* soil Nelson %	"Bush" Sick† soil Auckland Prov. %	"Waihi" Disease‡ soil Wairarapa %
Loss on ignition		6·1	—	12·66
Total nitrogen		0·10	0·238	0·286
Sol. in 1 % citric acid	Available P_2O_5	0·003	0·003	0·003
	Available K_2O	0·007	0·017	0·033
Sol. in HCl Sp. Gr. 1·1	Total P_2O_5	0·025	0·02	0·02
	Total K_2O	0·23	0·075	0·54
Lime requirement Fig.		0·32	0·375	0·36
*p*H value		5·1	—	5·3

* Average for 8 samples of soil from different parts of the Moutere Hills soil type.

† Average for data quoted by Grimmett and Simpson, *N.Z.I. Trans.* **59**, 401.

‡ Typical analysis quoted by B. C. Aston, *N.Z.I. Trans.* **59**, 650.

The chemical analyses of the three soils reported in Table I show certain striking resemblances. The soils are all highly deficient in available and total phosphoric acid. The lime requirement figures are similar, indicating a great need for lime treatment. The soils, however, differ considerably in their content of nitrogen and organic matter. In texture, the Moutere Hills soil is a loam while typical "Bush" sick soils investigated by B. C. Aston are sandy silts. The "Waihi" disease soils investigated by B. C. Aston in the Wairarapa are loams.

So far as the authors are aware no xanthin calculi have ever been reported from the "Bush" sick or "Waihi" disease areas. If the formation of the xanthin calculi is primarily due to phosphate starvation it seems remarkable that with soils showing such similar chemical criteria as those in the table, xanthin calculi should be of common occurrence

on the one soil and absent in the other two. There is of course the possibility that the xanthin calculi occur in the other areas but have not been reported. It is worth recording that though the officers of the Cawthron Institute have been in constant association with the farmers on the Moutere Hills for nearly 10 years, it has only been during the last year that attention was drawn to the calculus occurrence. Enquiry then showed that the trouble was very widespread and of long standing. Mr B. C. Aston, Chief Chemist to the Department of Agriculture, who has made a special study of "Bush" sickness and "Waihi" disease has kindly undertaken to have a careful search made for calculi in the areas where these diseases occur.

As might be expected from the high lime requirement figure and low content of phosphate, heavy liming and phosphatic manuring are essential for most crops on Moutere soil.

The many experiments conducted by the Cawthron Institute have shown that excellent yields of several crops with increases exceeding 1000 per cent. can be obtained by the use of 2 tons of ground limestone and 5 cwt. of phosphatic manure per acre. Where great exhaustion of soil has taken place English grasses and clovers cannot be established without the liberal use of both lime and phosphate. Bacterial counts made by Mr W. C. Davies at the Cawthron Institute show that the untreated Moutere soil has an abnormally low bacterial population and that where good crops have been established by lime and phosphate treatment a corresponding increase in bacterial population has occurred.

THE PASTURES ON MOUTERE HILLS SOIL.

Farmers state that in the early days of the settlement of the Nelson district, after the bush was burnt, a good sole of English grasses and clovers was obtained on many farms of the Moutere Hills soil.

In those days two sheep per acre could be carried and many fat sheep were sent to the market. At the present time the carrying capacity over large areas has been reduced to half sheep or less per acre. On a considerable number of farms not more than one-third sheep per acre can be maintained.

The English grasses and clovers have long ago disappeared, being replaced by Danthonia, sweet vernal and *Agrostis* sp.

Some lotus sp., trefoil and a little white clover can be found on farms where careful stocking and management have been observed.

Over large areas of poor hill land bracken has taken possession.

Tea-tree (*Leptospermum* sp.) and in places blackberry have a good hold on certain parts of the Moutere Hills.

As far as the investigation has gone the highest mortality of stock and also the greatest incidence of calculus trouble have occurred on farms where great deterioration in pasture has taken place.

Chemical examination of Moutere Hills pastures.

During the autumn of 1928, five samples of pasture were collected from different parts of the Moutere Hills soil. The samples were taken from areas which had been burnt over during the summer, and consisted entirely of young green growth from fairly extensive pastures. Little selection of individual grasses or areas by the grazing sheep was noted.

The average mineral content for the five samples is shown in Table II. The analytical data for spring pasture samples collected from particular areas where heavy loss of sheep has been experienced are also given. For comparison the average mineral content of a number of good Nelson pastures is included in the table.

Table II. *Analysis of Moutere Hills pastures.*

	Moutere Hills autumn growth (av. 5 samples)	Moutere Hills* spring growth	Moutere Hills† spring growth	Good Nelson pastures autumn growth (average)
Lab. No....	—	113	123	—
CaO	0·61	0·49	0·56	0·80
P_2O_5	0·63	0·56	0·63	1·06
K_2O	3·19	1·64	—	3·81
Na_2O	0·12	0·31	—	0·49
Cl	1·07	0·42	—	1·45
N	3·43	2·01	—	5·23
S	0·33	0·22	—	0·42
Fe	0·011	0·030	0·044	0·026
Mn	0·052	0·016	0·018	0·019
Total ash	11·67	10·40	—	11·51
Sol. ash	6·15	3·81	—	9·02
Insol. ash	5·52	6·59	—	2·49

Note. All determinations expressed as percentages of dry matter.

* Collected from typical hill country where much calculus trouble in sheep has occurred.

† Collected from typical hill pasture where both sheep and cattle eat bonemeal "lick" greedily. Great loss in hoggets in certain seasons has been experienced on this farm. Calculi in the kidneys of sheep have been found but mortality accompanied by this trouble is not so high as is the case on pasture represented by sample No. 113.

The outstanding features of the analytical data are: (1) The low content of nitrogen and soluble ash in the Moutere Hills samples. This is particularly marked in the spring sample No. 113. (2) The low lime and phosphate content of all samples from the Moutere Hills. This is

again very apparent in the case of sample No. 113. (3) The low iron and high manganese content of the autumn samples from the Moutere Hills. In the spring samples the percentages of iron and manganese are apparently normal. The abnormal figures for iron and manganese in the autumn samples may have been induced by the exceptionally dry summer and autumn experienced in 1928. Samples of autumn growth collected from certain other soil types in 1928 also gave exceptionally low figures for iron. A large increase in the iron content of the spring samples from these types has been likewise found. Although the percentage of iron in the autumn samples of Moutere Hills pasture is as low as that found by B. C. Aston[1] in the "Bush" sick pastures of the Rotorua district, typical symptoms of "Bush" sickness do not occur in stock grazing on Moutere Hills pastures. The great increase in the iron content of the spring pasture samples suggests that in months of normal rainfall the pastures of the Moutere Hills contain an adequate supply of iron. If such a low percentage of iron is a normal feature of Nelson pastures in the autumn it seems possible that the health of stock may be adversely affected during this period of the year.

The great deficiency of both lime and phosphate in Moutere Hills pastures is revealed in all the analyses which have been made. The deficiency is strikingly apparent on the pasture of one farm where calculi in the kidneys of sheep frequently occur. The percentage of lime falls considerably below that found by B. C. Aston in the Mairoa[2] pastures, which have been shown by him to be deficient in lime. There can be little doubt that the low content of both lime and phosphate in the Moutere Hills pastures is closely connected with the poor stock results obtained on many farms.

EXAMINATION OF MILK FROM COWS ON MOUTERE HILLS PASTURE.

The analytical data for some mineral constituents in the milk of a cow grazed solely on Moutere Hills pasture are shown below.

Table III.

	Milk (Moutere Hills)	Milk (normal)*
Ash	0·80	0·72
CaO	0·17	0·161
P_2O_5	0·213	0·189

* Quoted by W. Godden, *Agricultural Progress* (1928), **5**, 1.

[1] *Trans. N.Z. Institute*, **59**, 406. Mr Aston has shown that the "Bush" sick pastures are deficient in iron and that an iron ration is a remedy for the disease.

[2] *Ibid.*

The animal giving the milk was in poor condition. The milk yield was low. The cow eagerly devoured a "lick" of bonemeal and salt whenever access to the "lick" was permitted. The results shown in Table III agree with those of other workers in showing that the mineral composition of milk is little affected by an exclusive diet of a mineral-deficient fodder. In the case of other cattle on the farm where the sample of milk was obtained great improvement in appearance and vigour of the animals was noticeable after several weeks' feeding with a little bonemeal every day.

Examination of the blood of sheep affected with urinary calculi.

Some of the analytical data in connection with the blood of two sheep badly affected with calculi are given in Table IV.

Table IV. *Analysis of whole blood of sheep affected with xanthin calculi.*

	Sheep (1)	Sheep (2)
P_2O_5	0·0356	0·0340
CaO	0·0099	0·0111
MgO	0·0080	0·0080
K_2O	0·0624	0·0508
Na_2O	0·363	0·399
Cl	0·311	0·323
N	2·663	2·044
S	0·132	0·138
Fe	0·051	0·031
Mn	Nil	Nil
Total ash	0·861	0·930
Sol. ash	0·844	0·916
Insol. ash	0·017	0·014
Total solids	17·40	13·80

All results as percentages of whole blood.

Note (1). Sheep 1 yielded much more blood than Sheep 2.

Note (2). Abderhalden, quoted by Robertson in *Principles of Biochemistry*, gives the following data for the blood of healthy sheep: total solids = 17·54 %, iron (Fe) = 0·038 %.

Sheep No. 1 was a 2-year-old ewe—a Romney cross. It was bred on the property and weighed 52 lb. when killed. Numerous small stones were found in the kidneys. Sheep No. 2 was a 2-year-old wether—a Merino cross. It was bred on the property and weighed 49 lb. when killed. Both kidneys of this sheep were greatly enlarged and contained several large calculi as well as numerous small stones and coarse powder. The sheep was anaemic in appearance and outwardly exhibited all the symptoms of chronic kidney trouble. The most striking features of the analytical data presented in Table IV are the low content of total solids,

nitrogen and iron in the blood of sheep No. 2. This is in keeping with the observed anaemic condition of the sheep and suggests the possibility that the low iron content of Moutere Hills pastures already noted in the analysis of the autumn samples has a prejudicial effect on the health of sheep.

Owing to the paucity of reliable analytical data for the blood of healthy sheep, it is not possible to draw definite conclusions concerning deficiencies of minerals in the blood of the sheep affected with calculi. Theiler (11) and his colleagues have shown that a low content of both total and inorganic phosphate is noticeable in the blood of animals suffering from "styfsiekte." In certain of their experiments, however, no great reduction in the total phosphorus content of the blood occurred until two or three months before the actual death of the animals. Under these circumstances, the lack of any abnormality in the mineral composition of whole blood cannot be regarded as proof of the absence of serious deficiency in the diet of the animals.

EXAMINATION OF BONES FROM SHEEP AFFECTED WITH URINARY CALCULI.

The analysis of the leg bones from sheep (1) mentioned in Table IV did not reveal any significant differences from the data mentioned by other workers in connection with normal sheep. It must be remarked, however, that the sheep in question was under two years of age. Under these circumstances it is less likely that the bones would be highly deficient in either lime or phosphate. The bones of full grown cows and sheep on the Moutere Hills are, however, very thin and fragile.

FIELD EXPERIMENTS.

In order to determine the best means of overcoming the tendency for calculus formation in sheep on poor pastures on the Moutere Hills, several sets of experiments have been initiated. The experiments include (1) the value of basic slag alone for top-dressing pasture; (2) the value of basic slag with ground limestone for top-dressing purposes; (3) the value of bonemeal "lick" without resort to top-dressing of the pastures. Hoggets have been placed on the experimental blocks including control areas and the weights of the sheep are being recorded at regular intervals. The experiments have now been in progress nearly four months. All the sheep have increased in weight even on the control areas. The most significant features so far resulting from the experiments are: (1) Improvement in the flora of all top-dressed areas. The percentage of white

clover, lotus sp. and trefoil is already much increased. (2) Increase in the carrying capacity of all treated areas. So far the treated areas have successfully carried double and treble the number of sheep on the control areas. (3) Sheep and cattle are readily eating the bonemeal "lick" and are showing marked improvement.

The experiments have not proceeded long enough to enable conclusions to be drawn concerning the efficacy of the treatments in overcoming calculus formation.

Summary.

The occurrence of xanthin calculi in the kidneys of sheep on certain poor pastures on the Moutere Hills in the Nelson district is reported and described. The incidence of xanthin calculi is associated with poor stock results over large areas. Poor lambing returns, high mortality of stock in certain seasons, low milk yields and inability to fatten stock are common features of many farms on the Moutere Hills soil. On one small farm, grazing 120 sheep, no less than 12 sheep which have died or have been killed during the last six months have had calculi in the kidneys.

The Moutere Hills soil type is shown to be highly deficient in both lime and phosphate. Great increase in crop production invariably accompanies the application of lime and phosphate to the land.

The analysis of pasture samples from different parts of the Moutere Hills reveals a striking deficiency of both lime and phosphate even in the young growth. In more mature grass, the deficiency of these minerals should be still greater.

Samples of green growth, obtained after a dry summer and autumn, exhibit a great deficiency of iron and an abnormally high content of manganese. The content of iron and manganese in spring samples of green growth is normal. This suggests that a deficiency of iron is not the main factor leading to mortality of stock. Typical "Bush" sickness, moreover, a malady definitely associated with iron shortage, is not known on the Moutere Hills.

Field experiments have been initiated on farms where the sheep are known to be badly affected with calculi. The experiments have not proceeded sufficiently long to enable any conclusions to be drawn concerning the effect of top-dressing and the provision of "licks" on the incidence of calculus formation. Improvement in the flora of the pastures is already marked on top-dressed areas and a great increase in carrying capacity has been effected.

Fig. 1. Swollen sheep's kidney full of xanthin gravel.

Fig. 2. Single calculus from kidney pelvis; weight

Fig. 3. Kidneys of a badly affected sheep. (Notice fat shortage.)

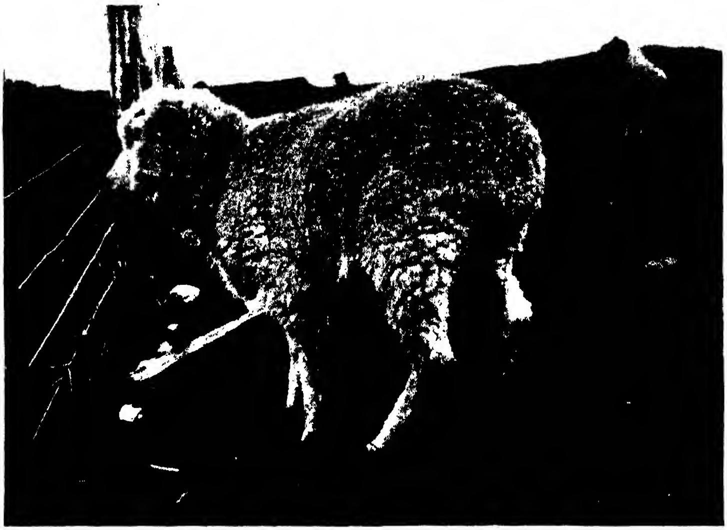

Fig. 4. Typical calculus-affected sheep.

Fig. 5. Two-year-old Moutere Hills bull ravenous for bonemeal "lick."

Calculus formation has not been noted on the small areas of Moutere Hills soil type where good pastures have been established by lime and phosphate treatment of the land.

Questions are raised with regard to the possible effect of mineral deficiency upon purin metabolism.

The authors acknowledge the valuable assistance given by many farmers during the course of the investigation. They are particularly indebted to Mr R. Davies, of Wakefield, who has given much valuable information and personal assistance. They are also indebted to Mr L. Bishop for assistance in the analysis of Moutere Hills soil samples and to Mr W. C. Davies for the photographs illustrating this paper.

The above investigation has been carried out at the Cawthron Institute in connection with the Empire Marketing Board's Scheme for pasture research throughout the Empire. The Empire Marketing Board and the N.Z. Research Council have contributed generously towards the cost of the investigation.

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NOTE ON THE EFFECT OF SODIUM SILICATE IN INCREASING THE YIELD OF BARLEY.

TO THE EDITORS OF *THE JOURNAL OF AGRICULTURAL SCIENCE.*

THE last issue of this *Journal* (p. 132) contains a paper by Dr R. A. Fisher on the effect of Silica upon the growth of Barley at Rothamsted, which begins by stating that his data "show conclusively that the view previously rejected that the silicate acts by making available to the plant the actual reserves of soil phosphates must be regarded as strongly established." Twice elsewhere Dr Fisher states that this erroneous conclusion of previous investigators is due to the fact that they had considered only the proportion of phosphoric acid in the ash and had overlooked the increase in the total phosphoric acid in the crop. As Mr Morison and I were the previous investigators in question I turned to our twenty-three-year-old paper with some curiosity to ascertain the grounds for this magisterial dismissal of our conclusions, for my remembrance of the subject did not tally with the opinion Dr Fisher attributes to us. Still less do I agree now that I have re-examined our original paper.

First as to the data. Dr Fisher has discussed statistically the yields and ash analyses of 14 years distributed between 1868 and 1911; Mr Morison and I employed the yields of the 41-year period then available and the ash analyses of two years, 1903 and 1904. The results are in agreement; Dr Fisher's analysis reveals no new facts. The question then turns upon the deductions we made from the agreed data.

So far from overlooking the total phosphoric acid removed in the crop I find Morison and myself expressly inviting our readers to consider "the whole amount of phosphoric acid removed by the crop...instead of the proportion of phosphoric acid in the ash." The prime fact disclosed by both our data and Dr Fisher's is that the silica has caused an increased intake of phosphoric acid. In our words "The silica acts by causing an increased assimilation of phosphoric acid by the plant, to which phosphoric acid the observed effects are due."

Morison and I suggest three possible explanations of this result:

(1) That the silica acts by enabling the plant to make fuller use of whatever phosphoric acid it had obtained from the soil.

(2) That the seat of the action is in the soil, where the silica

liberates or renders available to the plant more of the reserves of phosphoric acid in the soil.

(3) That the seat of the action lies in the plant itself, the silica "will stimulate the plant to assimilate a greater amount of phosphoric acid should that be available from the medium in which the plant is growing."

We dismiss (1), a view previously expressed by Wolf and Kreuzhage, on the ground that "the extra phosphoric acid derived from the soil is itself sufficient to explain the greater yield brought about by the silicate without attributing to the silica within the plant any specific action in economising the phosphoric acid there present."

Morison and I then proceeded to consider the alternatives (2) and (3), and preferred (3) "the seat of action is within the plant and not in the soil" because of evidence drawn from soil analyses and a water culture experiment. In the water cultures the barley took up more phosphoric acid (with attendant increase of growth) when silica was present, even though there remained an excess of phosphoric acid in the solution. We still regarded the evidence as insufficient and indicated the need for further experiments, which for various reasons were never completed. Mustard, a plant which contains little silica, was grown upon the plots and showed parallel results to those given by barley, *i.e.* an increased assimilation of phosphoric acid, a larger yield, and an earlier ripening. This result with a non-silica plant may be regarded as evidence that the action took place in the soil, but the other interpretation is not excluded.

We certainly never committed ourselves to the view that Dr Fisher attributes to us—that the function of the silica is one "of merely stimulating growth with the secondary effect that more phosphoric acid is absorbed." We wrote "Though the seat of the action is thus transferred from the soil to the plant, it is by no means settled whether the stimulus which the silica gives to the plant to enable it to take up more phosphoric acid from the soil reserves is a general stimulus or a specific one confined to the phosphoric acid. In other words, does the presence of a free supply of soluble silica so invigorate the plant that it is enabled to repair any link in the chain of nutrition and get as need be more nitrogen, phosphoric acid and potash from the soil, or is the beneficial action confined to the phosphoric acid alone?" The use of the word "stimulus" is not free from ambiguity, but it is evident from the above that our conclusions, admittedly tentative, went no farther than crediting silica with an action upon the plant rather than upon the soil. But on this point Dr Fisher's data throw no new light. His results only

confirm the results we reached in the early part of our paper, *i.e.* that silica brings about increased assimilation of phosphoric acid. They supply no additional evidence towards the interpretation; that will only arise from fresh experimental work. Possibly an explanation is to be sought in the colloid nature of the silica (see Comber, *J. Agric. Sci.* 1922, **12**, 365).

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THE DETERMINATION OF EXCHANGEABLE BASES IN SOILS.

MAGNESIUM, POTASSIUM AND TOTAL BASES.

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IT has been shown in an earlier paper (9) that semi-normal acetic acid may be used as a leaching reagent for the determination of exchangeable calcium in carbonate-free soils. As the energy of absorption of calcium is greater than that of the other exchangeable bases, magnesium, potassium and sodium, it may be assumed that these bases are totally removed by any treatment which removes the calcium. In order to verify this supposition, the writer has estimated the magnesium and potassium in some acetic acid extracts for comparison with the amount extracted by *N* ammonium chloride and, in some cases, *N* sodium chloride solutions. The leachings were carried out in the ordinary way, a litre being collected, of which half was used for the estimation of magnesium and half for the estimation of potassium. Before presenting the figures it may be desirable to outline the methods used for estimation.

ESTIMATION OF MAGNESIUM.

The calcium was precipitated by excess of ammonium oxalate. After filtering and washing, the magnesium was precipitated in the filtrate by means of sodium ammonium phosphate. After standing for 24 hours, the precipitate was filtered, thoroughly washed, redissolved in dilute nitric acid, and reprecipitated by sodium ammonium phosphate. It was then collected and estimated in the usual manner as magnesium pyrophosphate. This double precipitation was resorted to in all cases, as the presence of salts such as ammonium oxalate tends to cause too high results to be obtained for magnesium. In all cases blank experiments were carried out with the A.R. reagents employed, and, where necessary, corrections applied to the figures.

ESTIMATION OF POTASSIUM.

The potassium was estimated in the extract, after certain preliminary treatments, by the cobaltinitrite method using the technique developed by Milne (6), who introduces some modifications in detail to the method

as described by Mitscherlich (7) and Christensen (1). The preliminary treatment consisted in the removal of the excess of extracting reagent, acetic acid or ammonium chloride, and the rendering insoluble of silica, sesquioxides and phosphates. The acetic acid was easily removed by evaporation with a little hydrochloric acid, and the ammonium chloride by adding about 3 gm. of concentrated nitric acid for each gram of the salt present in solution and gently boiling (2). The other substances were rendered insoluble by evaporation to dryness and gentle ignition for several hours, finally extracting with water and filtering. Blank experiments were carried out simultaneously on the reagents. The figures obtained for magnesium and potassium are shown in Tables I A and I B.

Table I A. *Comparisons of amount of magnesium extracted from soils by different leaching solutions.*

Soil	Mg. equivalents per 100 gm. soil		
	N NaCl	0·5 N HAc	N NH_4Cl
LR 59	3·06	2·89	—
B 47	2·55	2·59	—
R 50	1·83	1·82	1·72
C 291	3·34	3·32	3·53
D 146	—	2·35	2·00
D 145	—	4·68	5·32
BO 4	—	1·0	0·6
F 43 B (2) ...	—	3·53	3·53

Table I B. *Comparisons of amount of potassium extracted from soils by different leaching solutions.*

Soil	Mg. equivalents per 100 gm. soil	
	0·5 N HAc	N NH_4Cl
R 50	0·25	0·285
D 146	0·32	0·285
C 291	0·214	0·214
D 145	0·36	0·36
BO 4	0·214	0·214
F 43 B (2) ...	0·15	0·25

The agreement between results obtained with different extracting reagents is seen to be very close in the majority of cases, and is quite satisfactory, especially in view of the fact that, in some of the soils, such small quantities are being estimated in the presence of so large a bulk of reagent materials. These figures, together with those presented for calcium in the previous paper (*loc. cit.*), may therefore be considered sufficient evidence for concluding that the category of bases removed by leaching with acetic acid is identical with that removed by leaching with N sodium or ammonium chloride solutions. Comparisons of the amount

of sodium extracted by the different reagents were not made as the content of exchangeable sodium in the soils under examination was generally of smaller magnitude than the potassium, and such determinations, if made, would be loaded with the errors of the other determinations. In view of the results for the other exchangeable bases it may, therefore, be assumed that a similar agreement would be obtained.

DETERMINATION OF THE TOTAL EXCHANGEABLE BASES.

Hissink (3) has shown that, in Dutch clay soils, calcium forms approximately 80 per cent. of the total exchangeable bases. This is the figure generally assumed for the purpose of calculating the total content (Hissink's *S*-value) when only the percentage of calcium is known. It is obvious that, whilst this relationship may hold for certain types of soils, it may vary considerably in others and that the only certain method of computing the total content of exchangeable bases is by direct determination. The results are most conveniently expressed as milligram equivalents per 100 gm. of soil. This determination entails a very considerable expenditure of time and reagent. Methods have been devised which give, at least, an approximate figure, for example, the method of Spurway (8) for the determination of active base and a recent method of Kappen (5). In both these methods soil is shaken up with a known volume of standard hydrochloric acid and the excess acid afterwards titrated with standard alkali. An objection can be put forward against these methods that the acid may, in certain soils, attack and dissolve bases from the unweathered mineral particles, thus giving a figure for the total exchangeable bases in excess of the correct figure. Further, in neither of these methods is the reaction carried to completion by leaching. The former objection does not appear to hold in the case of acetic acid. However, it would be necessary to use acid of greater strength than 0·1 *N*, used by Kappen for hydrochloric acid, and the errors in the titration of one litre of leachings would be too great for even approximate determinations.

Leaching with acetic acid brings into solution all the exchangeable bases of the soil as acetates, and this suggested to the writer a method by which their total in mg. equivalents might be determined. By ignition of alkaline and alkaline earth acetates, oxides and carbonates are formed. These oxides and carbonates would be expected to be quantitatively soluble in dilute hydrochloric acid. Thus, if an acetic acid soil extract were evaporated to dryness, ignited, and treated with a known volume of standard hydrochloric acid, the total of the bases

39–2

present should be determinable by titration of the excess acid with standard alkali. Before attempting any determinations on actual soil extracts, it was considered advisable to test the method by working with pure solutions of acetates of known composition. Further, as other substances, *e.g.* iron and aluminium, are removed from the soil by acetic acid, the effect of adding iron and aluminium acetates, and acetic acid leachings obtained from soils previously leached with the acid to remove the exchangeable bases, was investigated.

Estimation of acetate solutions.

A solution of mixed acetates was prepared by dissolving calcite, magnesium oxide, sodium carbonate, and potassium carbonate (A.R. materials) in 500 c.c. of acetic acid, the amount of calcium being approximately five times that of each of the others. The reaction value (*i.e.* the total of the bases expressed as mg. equivalents) of this solution was 1·864 mg. equivalents per 25 c.c. In the first series of experiments the following procedure was adopted. 25 c.c. lots of the solution were evaporated to dryness in small porcelain dishes on a water bath and then placed in a steam oven until perfectly dry. When dry they were carefully ignited in the muffle furnace or over a Bunsen burner. They were then allowed to cool and 20 c.c. of 0·2 *N* hydrochloric acid added to each one, the solid material being well rubbed in the acid with a rubber tipped glass rod, and the dishes covered with a clock glass and allowed to stand for a time. After contact with the acid no residue was found in some cases, but a certain amount of carbonaceous material was obtained in others. This was due to the difference in the time and intensity of ignition, but in this series of experiments it did not interfere with the subsequent titration of the excess acid. After standing for a definite period of time the contents of the dishes were washed into a conical flask and the excess acid titrated with standard sodium hydroxide. The results of these experiments are shown in Table II. The first twelve figures were of a preliminary character and methyl orange was used as indicator for the titration of the excess acid. The last six figures were under standardised conditions suggested by the results of the first series, but for reasons given in the section dealing with the effect of iron and aluminium, phenolphthalein would have to be used as indicator when applying the method to soil extracts; this indicator therefore was used to obtain these figures and a specially prepared carbonate-free standard solution of sodium hydroxide was used for the titration.

Table II. *Estimation of mixed acetates in a solution of known composition.*

Ignition conditions	Time of contact with acid	Mg. equivalents in 25 c.c. Found	Theoretical
Bunsen	60 mins.	1·819	1·864
Fairly strong in muffle ...	<60 ,,	1·842, 1·746, 1·768, 1·803	,,
Strong in muffle	<60 ,,	1·800	,,
,,	60 ,,	1·822	,,
,,	90 ,,	1·889	,,
,,	3 hours	1·867	,,
,,	Overnight	1·854	,,
Gentle in muffle	,,	1·882, 1·888	,,
Dull red, muffle for 5 mins.	,,	1·839, 1·839, 1·840, 1·846, 1·840, 1·834	1·837

A consideration of the figures shows the necessity of allowing the acid and the ignited residue to remain in contact for a period exceeding one hour. Rejecting those with one hour, or less, contact with the acid, the remainder of the first twelve show a maximum deviation from the theoretical of 0·025 mg. equivalents, approximately 1·3 per cent. This deviation is considerably less in the last six figures obtained under the prescribed standard conditions, viz. 0·009 mg. equivalents, or approximately 0·5 per cent. The agreement here is sufficiently good to warrant the assumption that a mixture of these acetates can be accurately estimated in this manner. In all subsequent experiments the ignition was carried out at a dull red heat for about five minutes and the acid allowed in contact with the ignited residue overnight.

EFFECT OF IRON AND ALUMINIUM.

In the preceding section mention was made of the fact that small quantities of iron and aluminium are likely to be dissolved from the soil when leaching with acetic acid and these substances might interfere with the titration of the ignition residues of the other bases. The effect of these two metals was, therefore, examined by adding small amounts of their acetates to a standard solution of the other acetates. However, it was found necessary to modify slightly the procedure described in the preceding section because, at the outset, it was observed that the sesquioxides were not rendered completely insoluble by the ignition, and, therefore, some of the acid would be used up in dissolving them. But owing to the hydrolysis of the ferric and aluminium chlorides this acid can be titrated if phenolphthalein is used as indicator. The titration of the excess acid was, therefore, carried on until a faint pink colour with phenolphthalein persisted for about ten seconds on shaking the contents

of the titration flask. The figures given in Table III show that good agreement with the theoretical was obtained in these experiments and that the method can be used for the estimation of the bases in the presence of iron and aluminium.

Table III. *Estimation of mixed acetates solution of known composition in the presence of iron and aluminium.*

Amount of iron and aluminium present during estimation Mg. equivalents	Mg. equivalents: Ca + Mg + Na + K	
	Found	Theoretical
0·35, 0·35, 0·35, 0·35, 0·35	1·842, 1·841, 1·833, 1·818, 1·838	1·841
0·80	1·833	1·841

Estimation of mixed acetates solution of known composition in the presence of soil extracts.

Duplicate 25 gm. lots of soil were leached with 0·5 N acetic acid, collecting separately each consecutive 500 c.c. of the leachings. In one of the duplicates the reaction value of the bases in each 500 c.c. was determined by evaporating to dryness, igniting and treating with standard acid, etc., as previously described. The first litre of leachings obtained from the other duplicate was treated in a similar manner, but the subsequent 500 c.c. lots were mixed with a known volume of the standard acetate solution before evaporating to dryness. This was done in order to discover whether any substances present in the soil extract affected the estimation. It was found that the ignited residue from the soil leachings contained appreciable amounts of insoluble material after treatment with acid; therefore, the titration of the excess acid was carried out after filtering the contents of the dish and thoroughly washing the filter paper with boiled distilled water. The following figures were obtained from experiments on three soils.

Table IV A. *Estimation of the acetates in the soil extracts without addition of the standard acetate solution.*

		Exchangeable bases in successive 500 c.c. of leachings Mg. equivalents per 100 gm. of soil					
Soil	No.	1	2	3	4	5	6
F 43 B	(A)	5·64	1·46	0·46	0·17	0·03	—
	(B)	5·61	1·60	0·49	—	—	—
Aber M	(A)	2·02	0·10	0·04	Nil	0·01	Nil
	(B)	1·96	0·14	—	—	—	—
BO 6	(A)	5·60	0·80	0·09	—	—	—
	(B)	5·44	0·80	—	—	—	—

Table IV B. *Estimation of standard acetate solution in the presence of soil extracts.*

Soil extract		Amount recovered after allowing for amount present in duplicate (A) Mg. equivalents	Amount added Mg. equivalents
F 43 B	No. 4 (B)	1·836	1·841
„	No. 5 (B)	1·810	„
Aber M	No. 3 (B)	1·800	„
„	No. 4 (B)	1·800	„
„	No. 5 (B)	1·760	„
„	No. 6 (B)	1·772	„
BO 6	No. 3 (B)	1·832	„

These figures show that after the first litre the amount of bases brought into solution is small. Even in the case of F43 B—a heavy clay subsoil—it is only the equivalent of 0·013 per cent. CaO. It will be observed that the recovery of the added acetates of the standard solution is practically complete with the extracts from F43 B and BO 6 but not quite as satisfactory with the extracts from Aber M. This soil is a mountain soil rich in organic matter, the extracts being highly coloured as compared with the usual acetic acid soil extracts, and may be regarded as an extreme case in which interference with the estimation of acetates is likely to occur. The smallest recovery is about 96 per cent. which in a soil containing, say, the equivalent of 0·20 per cent. CaO would represent an error of less than 0·01 per cent. CaO. In a normal soil the error would be less than this figure. The last results were considered sufficiently encouraging to proceed with the estimation of the total exchangeable bases of a number of soils for the purpose of comparison with the figures obtained by separate determinations.

PROPOSED METHOD.

The following procedure was then adopted for estimating the reaction value of the bases extracted from soil by acetic acid. 25 gm. of soil are leached with 0·5 N acetic acid as described in the estimation of exchangeable calcium (*loc. cit.*). One litre of leachings is collected and 500 c.c. evaporated to dryness. The evaporation is most conveniently carried out during the first stages in a large porcelain dish, transferring, when the volume becomes small, into a small 9 cm. porcelain dish in which the subsequent operations may be carried out. When the evaporation is complete the dish is placed in the steam oven for a short time, as there is danger of spitting on ignition if the residue is not quite dry. The dish is placed in the muffle furnace at a dull red heat for about five minutes. When cold, 20 c.c. of 0·2 N HCl are added, the solid matter

being well rubbed in the acid with a rubber tipped glass rod. The dish is covered with a clock glass and allowed to stand overnight. The contents of the dish are then poured on to a small filter paper and washed well with boiled distilled water. The excess acid in the filtrate is determined by titration with standard carbonate-free sodium hydroxide, phenolphthalein being used as indicator. The titration is carried out to the point when the pink colour persists for about ten seconds on shaking the contents of the titration flask. Some error might be apprehended from the presence of carbon dioxide from the reaction of the 0·2 *N* hydrochloric acid with carbonates in the residue. The agreement shown in the tables indicates that the method used is applicable for routine purposes. For specially accurate work it would be preferable to include a short boiling before titration.

Comparison of total exchangeable bases by separate determination with proposed method.

Data obtained for the exchangeable bases by the proposed method were next compared with those obtained by separate determinations. For the six soils given in Table I figures were available for all the bases except sodium. This was now determined in acetic acid extracts by removing the calcium, the sesquioxides, silica, etc., converting the magnesium, potassium and sodium salts into sulphates and weighing the mixed sulphates, the sodium being calculated by difference. The

Table V. *Bases by separate determination and by determination of mixed sulphates.*

Soil	Mg. equivalents per 100 gm. Mg + K + Na Separate determination	Mg + K + Na Determination of mixed sulphates
R 50	2·70	2·96
D 146	3·10	3·05
C 291	3·625	3·54
D 145	5·25	5·0
BO 4	1·39	1·40
F 43 B (2)	3·85	3·54

mixed sulphates were now dissolved in water and the sulphate content estimated in the usual manner with barium chloride, the object of this last procedure being to determine whether the sulphate content corresponded to the reaction value of the sum of these three bases. If this proved to be the case, then the total of the bases for comparison with the proposed method could be computed from the exchangeable calcium

and the sulphate content of the other three bases, after converting to sulphate. The results obtained are given in Table V.

The agreement, though not perfect, seems sufficiently close to justify the use of the figure obtained from the mixed sulphates in subsequent comparisons. When comparisons with the proposed method and the sum of the bases were made (Table VI, columns (1) and (5)), it was immediately evident that in every case the proposed method showed a distinctly lower figure. In some cases the differences between the two methods amounted to more than 2 mg. equivalents. Such divergences cannot be accounted for by experimental errors. Therefore some other acid radicle which does not give an oxide or carbonate on ignition must be present. In soils the two likely radicles are chloride and sulphate, and should these be present they would appear in the acid extract in combination with some of the bases. On evaporation and ignition these salts would remain unchanged and would be unattacked by the 0·2 N HCl. The difference between the figures by the proposed method and those obtained by separate determination should, therefore, be accounted for by the sulphate and chloride present. In order to secure a fair comparison the base values of the chlorides and sulphates should be added to the value for the total bases by the proposed method. The amount of these two acid radicles in the acetic acid extracts of the soils under examination was now determined and was found to be appreciable in all cases. The figures are shown in columns (2) and (3), Table VI, and the sum of the base values of the chlorides, sulphates and acetates in column (4).

Table VI. *Comparison of total bases by proposed method and the total by separate determinations. Milligram equivalents.*

Soil	(1) Total by proposed method	(2) Chloride	(3) Sulphate	(4) Total 1+2+3	(5) Total by sum of bases
RYB	10·22	0·52	0·65	11·39	11·99
G 144	16·26	0·40	0·61	17·27	16·66
G 119	4·23	0·89	0·60	5·72	6·35
R 50	2·53	0·75	1·36	4·64	4·11
D 146	8·71	0·40	1·29	10·4	10·35
C 291	5·75	0·88	1·64	8·27	7·40
D 145	15·86	0·88	0·93	17·67	18·32
F 43 B	8·36	0·52	0·82	9·70	9·43
MP 3	11·37	0·40	1·78	13·55	13·82
G 192	2·52	0·16	1·2	3·88	3·55
BO 4	6·2	0·40	0·79	7·39	7·46
			Average ...	9·99	9·95

The results presented in this Table show that the total exchangeable bases by separate determinations correspond with the total determined by the proposed method plus the chloride and the sulphate present in

the acetic acid extract. When it is considered that the total bases by the two methods is arrived at by summation of the results of a number of experiments, each of which is subject to sampling and experimental errors, an exact agreement is hardly to be expected. The fact that variations occur in both directions, and that when the average of the two sets of figures is taken the difference is only 0·04 mg. equivalents per 100 gm. of soil in favour of the total by the proposed method, is sufficient to indicate that individual differences are due to experimental errors and normal variations in soil samples. It may be assumed therefore that the figure obtained by the addition of the base value of the chloride and sulphate present in the acetic acid extract to the total by the present method corresponds with the total exchangeable bases obtained by separate determinations.

The question now arises whether the figure obtained by either of these methods corresponds with the *S*-value, *i.e.* the total of the soil exchangeable bases. Hissink (4), in his first paper on the determination of exchangeable bases, demands of any method for their estimation that it shall distinguish water soluble salts, insoluble carbonates, adsorptively bound bases, and acid soluble salts. The soils under consideration in the present instance are devoid of carbonates, and leaching with the dilute acid prescribed leaves the so-called acid soluble bases unattacked. The above results, however, show that in soils even of such a humid climate as North Wales appreciable amounts of water soluble salts may be present and these are estimated with the exchangeable bases. In order to know accurately the amount of each of the exchangeable bases, it would be necessary to remove the water soluble salts by preliminary leaching. The total of the exchangeable bases is, however, given by the figure obtained from the acetate residue as shown in column (1), Table VI. The writer submits that this is a stricter measure of the *S*-value than any figure obtained from the content of exchangeable calcium or from the laborious determination of total exchangeable bases by methods hitherto proposed, in which the base value of water soluble salts may be unsuspectingly reckoned.

Summary.

(1) Exchangeable magnesium and potassium can be determined in carbonate-free soils by the use of 0·5 *N* acetic acid as a leaching agent.

(2) A method is described for the determination of the total exchangeable bases present as acetates in the leachings.

(3) The results obtained by this method differ from those obtained

by summation of separate determinations by an amount equivalent to the sulphates and chlorides present.

(4) It is suggested that the total exchangeable bases by the proposed method gives a truer measure of the exchangeable bases than methods in which bases present as sulphates and chlorides are also reckoned in.

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A STUDY IN SAMPLING TECHNIQUE: THE EFFECT OF ARTIFICIAL FERTILISERS ON THE YIELD OF POTATOES.

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(With One Text-figure.)

Introduction.

In order to test the adequacy of a sampling method for estimating the yield of a crop of potatoes, use was made of the plots of a part of the Rothamsted Potato Experiment of 1928. This experiment was designed to give information as to the effect on yield of applying nitrogenous, potassic and phosphatic fertilisers in various quantities. There was a basal dressing of dung at the rate of 14 tons per acre, and further nitrogen was supplied as sulphate of ammonia at rates of 0, $1\frac{1}{2}$ and 3 cwt. per acre; potash at rates equivalent to 0, 1 and 2 cwt. of sulphate of potash per acre; and phosphate as superphosphate at 3 cwt. per acre. The experiment was of the "Randomised Blocks" pattern devised by R. A. Fisher (1), and consisted of 81 plots arranged in 9 blocks. The effect of phosphate was found by dividing each plot into two equal sub-plots, only one of which, chosen at random, received superphosphate. The arrangement is shown in the figure.

The variety was "Ally." The sets were planted on April 17th–19th, and the crop lifted on October 19th. Each of the 162 sub-plots was 1/90th of an acre in area, consisted of three bouts 3·4 links apart and 108 links long, and included about 180 plants.

Three blocks of the experiment (*A*, *B* and *C* of the figure), comprising 54 sub-plots in all, were first sampled by the method described below, and then lifted with a Howard's "Spinner," followed by harrows, and weighed on the field, in order to find the total yield from each sub-plot.

The statistical problem.

It was pointed out in an earlier paper (2) that a valid estimate of error of sampling can only be made if the constituent parts of a sample are located independently and at random. These constituent parts need not necessarily be individual plants or single short lengths of drill, but

may be complex patterns as in the "échelon" method used by Engledow (5), provided that a random selection can be made from a "population" of patterns.

To avoid confusion the following terms will be used in the strictly technical sense indicated:

(a) "*Units*"—the ultimate parts of a sample. For cereals, short lengths of drill, or small areas; for roots and potatoes, individual plants, etc.

		A			*B*			*C*		
	3 O	6 P	9 O	9 P	6 P	5 O	2 O	9 P	4 O	
	3 P	6 O	9 P	9 O	6 O	5 P	2 P	9 O	4 P	
	1 O	7 O	2 O	8 O	4 O	1 O	7 O	8 P	5 P	
	1 P	7 P	2 P	8 P	4 P	1 P	7 P	8 O	5 O	
	4 O	8 P	5 O	7 O	2 P	3 O	1 P	3 O	6 P	
	4 P	8 O	5 P	7 P	2 O	3 P	1 O	3 P	6 O	
	2 P	7 P	8 O	5 O	9 P	6 O	7 P	2 O	4 P	
	2 O	7 O	8 P	5 P	9 O	6 P	7 O	2 P	4 O	
	3 O	6 O	5 O	1 O	4 O	7 O	5 P	8 P	9 O	
D	3 P	6 P	5 P	1 P	4 P	7 P	5 O	8 O	9 P	*F*
	4 O	9 O	1 O	2 O	3 O	8 P	3 P	1 P	6 P	
	4 P	9 P	1 P	2 P	3 P	8 O	3 O	1 O	6 O	
	9 O	6 P	7 P	3 P	9 P	8 P	2 O	6 O	1 P	
	9 P	6 O	7 O	3 O	9 O	8 O	2 P	6 P	1 O	
	2 O	8 P	4 P	4 O	1 O	7 P	3 O	5 O	8 O	
	2 P	8 O	4 O	4 P	1 P	7 O	3 P	5 P	8 P	
	1 O	3 P	5 P	2 P	6 P	5 O	4 O	9 O	7 O	
	1 P	3 O	5 O	2 O	6 O	5 P	4 P	9 P	7 P	
		G			*H*			*I*		

Key to treatments		
No.	Sulphate of ammonia cwt. per acre	Sulphate of potash cwt. per acre
1	0	0
2	$1\frac{1}{2}$	0
3	3	0
4	0	1
5	$1\frac{1}{2}$	1
6	3	1
7	0	2
8	$1\frac{1}{2}$	2
9	3	2

P—3 cwt. superphosphate per acre.
O—No superphosphate.

Fig. 1.

(b) "*Sampling-units*"—those parts of a sample which are located independently and at random within the area to be sampled. Each may consist of one or many "units."

(c) "*Sample*"—the aggregate of sampling-units taken from the area. A valid estimate of error is only obtainable if each sample consists of at least two sampling-units.

The interrelations of these parts determine three important characteristics of the sampling-error:

(a) *Validity*—at least two sampling-units, defined as above, are

necessary to provide a valid estimate of error. The number and distribution of units within the sampling-units play no part whatever in determining the validity of the estimate.

(*b*) *Magnitude*—*i.e.* accuracy of the estimate of yield—the magnitude of the error may be reduced either by increasing the number of sampling-units in the samples, or by adjusting the number and distribution of units within the sampling-unit. Care in spacing the units within large sampling-units will tend to make the latter individually representative of the area sampled, and therefore like one another—*i.e.* the sampling-error will be small. There is, however, a further point to be considered—the units should be located in such a way as to minimise the actual labour of sampling. This is of considerable importance where many plots have to be sampled, and provides a strong case for a simple systematic distribution of units.

(*c*) *Accuracy*—*i.e.* accuracy of the estimate of sampling-error—the relative accuracy is determined solely by the number of degrees of freedom on which the estimate is based; but, of course, the standard error of an estimate of the variance or standard deviation is proportional to the absolute magnitude of the value to be estimated.

Since the observations were made on a whole set of experimental plots (54), they differed essentially from those on cereals (2), where individual plots were dealt with. For assessing the yield of a single plot it is necessary to take a fairly large number of sampling-units, the actual number, of course, depending on the size of the plot and the uniformity of the crop, but rarely being less than 10. If fewer are taken the estimate of error is too inaccurate to be of much value. When a large number of plots is being sampled, however, as in the present case, each plot may be made to contribute to the estimate of error, if it can be assumed that the variation from sample to sample is sensibly the same on different plots. There is a considerable body of agricultural data to justify this assumption for plots bearing the usual English crops manured according to current practice, though it is certainly not true over the large range of manurial treatment given in pot-experiments, where the heaviest application may be twenty or thirty times the lightest. The experiment under notice being a field trial in which additional nitrogen was given at rates not exceeding 0·6 cwt. per acre, potash and phosphate being given at still smaller rates, it was satisfactory to assume that the variance was constant over all treatments. Under the circumstances it became possible to sample only in duplicate—*i.e.* to take only two sampling-units from each of the 54 sub-plots, the sampling-unit being a complex

structure designed to be highly representative of the plot, yet easily and rapidly taken.

Now we are dealing with a crop in which the individual plants are quite large and at some distance apart. The considerable variability usually present in the spacing of the plants makes a small metrical unit undesirable: greater lengths of bout would give unduly large units. The individual plant thus becomes the logical unit.

In the present case duplicate sampling-units were taken of every twentieth plant on each plot, the starting-points being selected at random from among the first twenty plants. It is easy to see that there are 190 different ways of choosing a pair of sampling-units from any one plot, so that if there are n plots, the method envisages a population of $190n$ pairs of sampling-units from which n are chosen at random. The conditions for a valid estimate of error are therefore fulfilled. Further, since there were 54 sub-plots, a direct estimate is based on $54 \times 1 = 54$ degrees of freedom, and is therefore well-founded.

THE FIELD TECHNIQUE OF SAMPLING.

The field technique of sampling was as follows: from a note-book containing a list of pairs of "starting-points" chosen at random was taken the pair of numbers which had been assigned to the sub-plot about to be sampled, and a white wooden peg was stuck in the ground beside the plant corresponding to the smaller of the two numbers. Similar white pegs marked every twentieth plant from this starting-point. The total number of pegs was noted, and also the number of plants beyond the last peg. This gave sufficient data for calculating the number of plants in the sub-plot. A black peg was then placed beside the plant corresponding to the larger of the two numbers, and at every twentieth plant from it. There were thus two sets of pegs marking the constituents of the two sampling-units to be taken from the sub-plot.

Each marked plant was lifted with a fork, care being taken not to disturb neighbouring plants, and the tubers rubbed to remove some of the adhering soil. They were then put in a basket and taken to the balance (placed on a table in the middle of the plot) where their total weight was found to the nearest ounce. The peg marking the plant was placed in the basket with the tubers, so that there was little danger of entering the weight wrongly in a note-book whose pages were divided into two columns, one for "black" and one for "white" sampling-units.

DETERMINATION OF THE SAMPLING-ERROR.

For the two sampling-units selected and weighed on each plot the following calculations were performed: (*a*) and (*b*) mean weight of a plant in ounces; (*c*) difference between mean plant-weights for duplicate sampling-units, *i.e.* (*a*) $\sim$ (*b*); (*d*) and (*e*) sum of squares (in ounces^2) of the deviations of individual plant-weights from the mean of the sampling-unit, and (*f*) the sum (*d*) + (*e*). A specimen page from the note-book is set out herewith, the plot selected being the half of B4 treated with superphosphate.

Table I.

	Weight in oz. 1st sampling-unit	Weight in oz. 2nd sampling-unit
Plot B 4 P	23	18
	16	15
	19	17
	17	22
	20	22
	12	18
	12	25
	18	31
	23	
Number of plants ...	9	8
Mean weight in oz. ...	17·8	21·0
Difference (*c*)	3·2	
Sum of squares ...	132	188
Sum (*f*)	320	

Actually 52 plots out of the 54 in Blocks A, B and C were dealt with in this way. The remaining two plots were left out of the calculation owing to uncertainty as to whether certain plants had been correctly assigned to sampling-units 1 and 2 respectively. The mean plant-weight over all the 52 plots was 19·104 oz. The sum of squares of deviations of the means of duplicate sampling-units from their common mean is, for this plot, $\frac{1}{2}$ $(3{\cdot}2)^2$, with 1 degree of freedom. Leaving the factor $\frac{1}{2}$ to be introduced at a later stage, the total sum of squares of deviations between actual means is obtained by adding together the squares of all these differences (*c*), one for each plot. The total is 757, with 52 degrees of freedom. Dividing this total by 52 and then by 4, and taking the square root, we obtain the standard error of the mean of duplicate sampling-units. But in order to see if the complex sampling-unit used is appreciably better—*i.e.* more representative of the plot—than a sample of the same number of independently located units, we may also calculate the variance between the means of samples selected at random,

in the following way. Sum all the quantities (f), *i.e.* find the total sum of squares of deviations of each plant-weight from the mean of the sampling-unit in which it is contained. This amounts to 45,921. Divide by the number of degrees of freedom (one less than the number of plants for each sampling-unit). Grouping according to the number of plants we have

No. of plants	Frequency	Degrees of freedom
7	2	12
8	43	301
9	55	440
10	4	36
	104	789

On dividing 45,921 by 789 we are left with 58·2015. This is the variance between plants. That between random means, for comparison with the figure 14·558 obtained for the variance between actual means, is obtained by multiplying 58·2015 by

$$\frac{2}{104} \times \left\{\frac{2}{7} + \frac{43}{8} + \frac{55}{9} + \frac{4}{10}\right\},$$

i.e. by 1/4·27216. The following table shows the results reached up to this point.

Table II.

	Degrees of freedom	Sum of squares	Mean square
Between plants	789	45,921	58·202
Between random means ...	789	10,749	13·623
Between actual means ...	52	757	14·558
	841	11,506	13·681

The variances between actual and random means are not significantly different, so that there is no apparent gain by the systematic arrangement, and we may take the combined figure 13·681 as the best estimate of sampling variance. Dividing by 4 and taking the square root of the result, we find that the standard error of a 1 in 10 sample is 1·8494, or 9·68 per cent. of the mean plant-weight 19·104 oz.

Table III.

	Degrees of freedom	Sum of squares	Mean square
Between plants	1133	205,807	181·648
Between random means ...	1133	24,642·4	21·750
Between actual means ...	36	507·2	14·089

Mean plant weight = 37·086 oz.

Standard error of sampling = $\sqrt{\{14{\cdot}089 \div 4\}}$ = 1·877, or 5·06 per cent.

It is interesting to compare the figure 9·68 with the analogous figure obtained from an experiment carried out at Woburn. Two blocks of

eighteen plots each were here selected for testing the sampling method, and the above results (Table III) obtained in precisely the same way as has been described. An interesting feature of these results is that the systematic arrangement has shown itself definitely superior to purely random sampling, as is seen on comparing the variance "between random means" with that "between actual means":

	Variance	$\frac{1}{2}\log_e$ (variance)
Between random means	21·750	1·5398
Between actual means	14·089	1·3227

The difference between half the natural logarithms of the variances is 0·2171. Looking up R. A. Fisher's table of z(3) with $n_1 = 1133$ and $n_2 = 36$, we find that we are just about at the 5 per cent. significance level, *i.e.* only once in twenty times would such a difference occur in two sample estimates of the same variance.

In view of the significance of this difference the smaller value, 14·089, is used in calculating the sampling-error, which is then found to be 5·06 per cent. This result may be interpreted as showing that the Woburn soil was more heterogeneous within plots than was the soil at Rothamsted, where there was no difference between the variances, and where, consequently, an average value was used.

The two estimates of error, 9·68 per cent. at Rothamsted, and 5·06 per cent. at Woburn, are not comparable figures, in that the former is based on plots only 1/90th acre in area, while at Woburn the plots were 1/40th acre in area. Since, however, the sub-plots at Rothamsted were arranged in pairs whose members were similarly treated except that only one received superphosphate, it is possible to arrive at an estimate of sampling-errors over plots of 1/45th acre, an area very nearly that of the Woburn plots. This is effected as follows: the differences in mean root-weight between members of the adjacent pairs of sub-plots are found for each pair, and from these are calculated the mean difference due to superphosphate, and also the sum of the squares of deviations from this mean difference. This latter quantity may fairly be taken as arising from soil differences between adjacent sub-plots. (It would also include interaction between phosphate and other manurial treatments, but these are shown to be insignificant.)

The variance of duplicate sampling-units from the same sub-plot provides an estimate of errors due to sampling alone. If now we add to the corresponding sum of squares that due to soil-difference between adjacent sub-plots (both reduced to a sub-plot basis), we can arrive at an estimate of the variance of a similar sample distributed over the

double area. Halving the figure gives the variance of a sample twice as large, and from the double area—1/45th acre.

Table IV.

	Degrees of freedom	Sum of squares	Mean square	Standard error
Differences due to sampling within sub-plots	841	2876·5	3·420	—
Soil differences between sub-plots	26	161·44	6·209	—
Total	867	3037·9	3·5039	—
			1·7520	1·324 or 6·93 %

It is noteworthy that plant number contributes a very much larger share to the total variation than does soil-heterogeneity. For on the whole plot of 1/45th acre the sampling variance of a 1 in 10 sample is little more than half that on 1/90th acre. (Doubling the plant number, if there were no soil effects, should halve it exactly.) This being so it is possible to estimate the corresponding figure for 1/40th acre, by neglecting the soil-factor. The variance, 1·7520, must now be multiplied by 8/9, giving 1·577 as the new figure. Hence the standard error becomes:

$$\sqrt{1{\cdot}577} = 1{\cdot}248, \text{ or } 6{\cdot}53 \text{ per cent.}$$

This figure is now directly comparable with the Woburn result of 5·06 per cent. Comparing the variances in units of 1 per cent.:

At Rothamsted $6{\cdot}53^2$ or 42·64,
At Woburn $5{\cdot}06^2$ or 25·60,

it is seen that the sampling is considerably more accurate at Woburn than at Rothamsted. This is probably due to the much greater percentage at Rothamsted of plants suffering from Leaf Roll, leading to a bigger variation in root-weight from plant to plant.

STATISTICAL TREATMENT OF ACTUAL AND ESTIMATED YIELD FIGURES.

Table V.

Actual yields in half lb.

	A			*B*			*C*		
	1	2	3	1	2	3	1	2	3
	391	521	480	531	564	454	503	563	494
	401	402	493	449	512	509	459	526	491
	4	5	6	4	5	6	4	5	6
	284	299	395	384	361	337	365	459	453
	278	349	438	427	365	395	420	460	502
	7	8	9	7	8	9	7	8	9
	283	426	411	318	410	420	312	383	565
	290	370	387	286	360	412	289	420	543
Block totals		6898			7494			8207	

The total produce from each plot was weighed and the weight of the samples previously taken from the plots added in, the total being recorded to the nearest half pound. Table V shows the serial numbering of the plots, for comparison with Fig. 1, and the actual yields in half pounds.

Table VI.

Treatment totals in half lb.

Totals of 3 plots			Amount of S/Amm. in cwt. per acre: 0	1½	3	Total	
Amount of potash in cwt. per acre S/Pot.	0	P	985	1307	1233	3,525	
		O	910	1258	1194	3,362	
	1	P	1146	1349	1650	4,145	
		O	1138	1367	1457	3,962	
	2	P	1055	1312	1587	3,954	
		O	982	1214	1455	3,651	
Total ...		P	3186	3968	4470	11,624	Phosphate total
		O	3030	3839	4106	10,975	No phosphate total
Grand total ...			22,599		General mean ...	418·5	

ANALYSIS OF VARIANCE. AGRICULTURAL YIELDS.

The 53 degrees of freedom may be analysed in the first instance into 2 for differences between different blocks of land, 17 for differences between treatments, and 34 on which to base an estimate of error. Of the 17 degrees of freedom 8 are accounted for by the nitrogenous and potassic treatments, and are obtained (see Table VI) from the nine totals of adjoining phosphate and no phosphate sub-plots (2 for nitrogen, 2 for potash and 4 for the interaction of these fertilisers). We then find that 16 (8×2) degrees of freedom out of the 34 for error are appropriate for furnishing an estimate of the error of the nitrogen-potash comparisons, while the remaining 18 are to be used for the phosphate comparison. The remaining 9 degrees of freedom for treatment consist of 1 for the direct comparison phosphate *versus* no phosphate, and 8 for the phosphate interactions with nitrogen and potash. The analysis is as follows:

Table VII.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$ (mean square)
Blocks	2	47,723·44	23,861·72	—
Nitrogen	2	160,967·44	80,483·72	5·6479
Potash	2	41,776·44	20,888·22	4·9735
Interaction	4	21,432·78	5,358·20	4·2932
Error (a)	16	49,662·89	3,103·93	4·0202
Phosphate	1	7,800·02	7,800·02	4·4809
Phosphate interactions ...	8	5,456·82	682·10	3·2626
Error (b)	18	14,227·67	790·43	3·3363
Total	53	349,047·50	—	—

It will be noticed that the error for the phosphate comparison is significantly smaller than the other, as we have a right to expect, for the treatments compared were on two adjacent plots throughout, and soil-differences would be much smaller than between plots separated by larger distances. In fact the correlation between adjacent sub-plots was + 0·59. Now examining the last column of the table, where half the natural logarithm of the variance is given, we find from the table of z, (i) that the effect of nitrogen and of potash is undoubtedly significant—not once in a hundred times would such a large difference occur in two sample estimates of the same variance—while the interaction of potash and nitrogen is not significant, (ii) that phosphate shows an undoubtedly significant effect—the probability of a difference of 1·1446 is less than 0·01—while the phosphate interactions are not significant.

We may therefore present our results in the form of two tables, one showing the straight comparison between the plots receiving and not receiving phosphate, *i.e.* between the means of 27 sub-plots, while the other shows the nitrogen and potash effects, and is obtained by averaging in each case the two sub-plots shown in Table VI. The standard errors appropriate to these comparisons are:

Phosphate comparison.

Standard error (mean of 27 plots) $= \sqrt{\{790{\cdot}43 \div 27\}}$
$=$ 5·41 half lb.;

or 1·29 per cent. of the mean yield 418·5 of the sub-plot.

Nitrogen and potash comparisons.

Standard error (mean of 6 plots) $= \sqrt{\{6207{\cdot}86 \div 6\}}$
$=$ 32·17 half lb.;

or 3·84 per cent. of the mean yield 837 of the whole plot.

Expressed in tons per acre, and as a percentage of the mean yield, the results are as follows:

Table VIII. *Summary of results—Actual yields.*

Average yield	Without superphosphate	With superphosphate	Mean	Standard error
Tons per acre ...	8·17	8·65	8·41	0·11
Per cent. ...	97·1	102·9	100·0	1·29

		Average yield in tons per acre, S/Amm. 0	1½	3	Per cent., S/Amm. 0	1½	3
Potash	0	6·34	8·59	8·13	75·5	102·2	96·7
	1	7·65	9·09	10·40	91·0	108·2	123·7
	2	6·82	8·46	10·19	81·1	100·6	121·1
		Standard error ...		0·32	Standard error ...		3·84

The principal conclusions from the experiment are: There is a significant response to the nitrogenous, potassic and phosphatic fertilisers. The response to phosphate was small, but was detected by the precise nature of the comparison made possible by the design of the experiment. There was no further response to the double dressing (2 cwt.) of potash, or to the double dressing (3 cwt.) of sulphate of ammonia in the absence of potash.

Estimated yields from sampling.

If the total produce had not been weighed, the yields would have had to be estimated by multiplying the mean plant-weight of the samples taken on any one plot by the total number of plants on that plot. The results, for comparison with Table V, are given below:

Table IX.

Estimated yields in half lb.

	A			*B*			*C*		
	1	2	3	1	2	3	1	2	3
	336	564	558	505	631	427	557	566	461
	369	399	565	440	579	500	508	528	487
	4	5	6	4	5	6	4	5	6
	253	292	327	341	437	337	372	386	360
	167	348	426	405	284	392	407	366	575
	7	8	9	7	8	9	7	8	9
	268	360	346	301	394	420	282	320	603
	251	345	400	286	366	449	286	426	549
Block totals		6574			7494			8039	

While the variations of the estimated and actual yields for individual plots are in some cases large, the treatment and block averages agree very well. It is a striking coincidence that the totals for Block B should be identical. The treatment totals are as follows:

Table X.

Estimated treatment totals in half lb.

Totals of 3 plots			Amount of S/Amm. in cwt. per acre: 0	1½	3	Total	
Amount of potash in cwt. per acre S/Pot.	0	P	841	1328	1244	3,413	
		O	876	1250	1076	3,202	
	1	P	1022	1260	1798	4,080	
		O	1166	1348	1527	4,041	
	2	P	1041	1151	1636	3,828	
		O	965	1052	1526	3,543	
Total ...		P	2904	3739	4678	11,321	Phosphate total
		O	3007	3650	4129	10,786	No phosphate total
Grand total ...			22,107		General mean ...	409·38	

There is a slight underestimate of the total crop yield, the mean 409·39 half lb. corresponding to a yield of 8·22 tons per acre, as compared with 8·41 tons per acre actual yield.

ANALYSIS OF VARIANCE. ESTIMATED YIELDS.

Table XI.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$ (mean square)
Blocks	2	60,919·44	30,459·72	—
Nitrogen	2	233,000·44	116,500·22	5·8328
Potash	2	63,001·33	31,500·66	5·1789
Interaction	4	69,753·90	17,438·48	4·8832
Error (*a*)	16	95,762·22	5,985·14	4·3485
Phosphate	1	5,300·46	5,300·46	4·2878
Phosphate interactions ...	8	22,221·37	2,777·67	—
Error (*b*)	18	54,129·67	3,007·20	4·0044
Total	53	604,088·83	—	—

Table XII. *Summary of results—Estimated yields.*

Average yield	Without superphosphate	With superphosphate	Mean	Standard error
Tons per acre ...	8·03	8·42	8·22	0·21
Per cent. ...	97·6	102·4	100·0	2·58

		Average yield in tons per acre S/Amm.			Per cent. S/Amm.	
		0	1½	3	0	1½	3
Potash	0	5·75	8·63	7·77	69·9	105·0	94·4
	1	7·33	8·73	11·13	89·1	106·2	135·4
	2	6·72	7·38	10·59	81·7	89·7	128·7
		Standard error ...		0·45	Standard error ...		5·46

Nitrogen and potash still show significant responses, while the variance due to interaction of these two fertilisers is larger than before, but does not quite approach the 5 per cent. level of significance. The phosphate effect, however, is insignificant. There is evidence from the agricultural yields that it exists, although only to a moderate extent. The larger standard error on the estimated yields, which necessarily includes the error due to sampling, has masked what was at best only a moderate response to the phosphatic dressing.

The standard errors are:

Phosphate comparison.

Standard error (mean of 27 plots) $= \sqrt{\{3007{\cdot}2 \div 27\}}$
$=$ 10·55 half lb.;

or 2·58 per cent. of the mean yield 409·4 of the sub-plot.

Nitrogen and potash comparisons.

$$\text{Standard error (mean of 6 plots)} = \sqrt{\{11970{\cdot}28 \div 6\}} = 44{\cdot}67 \text{ half lb.};$$

or 5·46 per cent. of the mean yield 818·8 of the whole plot.

The standard error appropriate to the phosphate comparisons is rather less than one-half that for the potash and nitrogen comparisons, as compared with one-third in the case of the actual yield figures. This indicates that there is some factor working against the favourable circumstance of the plots to be compared for the phosphatic effect being contiguous. The accuracy of the sampling method depends on the proportion of plants actually selected for weighing. This could be increased, but a point would ultimately be reached where there would be no substantial gain, either in time or economy of working, over the ordinary method of harvesting the whole crop. But given a certain sampling ratio, as the 1 in 10 of this experiment, the accuracy may be affected by differences in the numbers of plants on the several plots, or by an uneven crop. This field was admittedly patchy, the weight of tubers from individual plants sampled varying from 2 to 53 oz. The least satisfactory feature of the analysis conducted on the basis of the estimated yield figures from the samples is the obscuring of the effect of the phosphatic fertiliser by an increased standard error. To see how far this effect is due to variation in individual plant-weight, and how far to variation in plant-number from plot to plot, a corresponding calculation was made on the sampling plant-weight, *i.e.* the mean weight in oz. of a plant, estimated from duplicate sampling-units from the same sub-plot. Set out in half oz. units the figures are as follows:

Table XIII.

Sampling plant-weights in half oz.

	A			*B*			*C*		
	1	2	3	1	2	3	1	2	3
	32·6	51·3	53·1	46·2	58·7	40·9	52·1	53·6	43·4
	34·7	36·5	55·5	41·9	53·2	47·1	48·1	50·3	46·7
	4	5	6	4	5	6	4	5	6
	23·4	27·6	32·1	31·2	40·4	31·5	35·6	35·9	33·1
	16·8	34·8	40·1	38·8	27·4	37·1	40·4	34·2	54·1
	7	8	9	7	8	9	7	8	9
	24·8	34·1	33·0	29·2	38·0	40·0	28·6	30·5	58·1
	23·6	31·5	37·9	27·9	35·1	41·8	26·9	37·9	48·8
Block totals		623·4			706·4			758·3	

The actual number of plants per sub-plot may be obtained by dividing the figures in Table IX by those in Table XIII and multiplying

by 16. The numbers are fairly regular, varying from 158 to 180. In fact a cursory inspection of the two tables shows that the more important divergences from the actual yields of Table V are reflected in both, and are due, therefore, to the samples being in some cases insufficiently representative of the growing crop.

Table XIV.

Treatment totals in half oz.

Totals of 3 plots			Amount of S/Amm. in cwt. per acre: 0	$1\frac{1}{2}$	3	Total	
Amount of potash in cwt. per acre S/Pot.	0	P	82·5	126·2	114·4	323·1	
		O	81·8	119·3	103·1	304·2	
	1	P	97·7	118·1	168·1	383·9	
		O	108·6	128·0	138·5	375·1	
	2	P	103·1	108·8	155·3	367·2	
		O	92·4	96·9	145·3	334·6	
Total ...		P	283·3	353·1	437·8	1074·2	Phosphate total
		O	282·8	344·2	386·9	1013·9	No phosphate total

Grand total ... 2088·1 General mean ... 38·6685

ANALYSIS OF VARIANCE. SAMPLING PLANT-WEIGHT.

Table XV.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$ (mean square)
Blocks	2	514·46	257·23	—
Nitrogen	2	1857·74	928·87	3·4170
Potash	2	484·57	242·28	2·7450
Interaction	4	679·38	169·84	2·5674
Error (a)	16	913·25	57·08	2·0222
Phosphate	1	67·34	67·34	2·1049
Phosphate interactions ...	8	203·47	25·43	—
Error (b)	18	443·35	24·63	1·6020
Total	53	5163·56	—	—

The effect of the nitrogenous and potassic fertilisers shows up in very much the same way as it did in the analysis of the estimated yield figures, while that due to phosphate, though still not significant, is distinctly larger than before. It is interesting to note that the difference between the errors (a) and (b) is again significant, although not to the same marked extent as in the case of the actual yield analysis. This shows that the variation in plant-number between contiguous sub-plots was so large as partially to obscure the advantages known to follow from the arrangement.

The standard errors are:

Phosphate comparison.

Standard error (mean of 27 plots) $= \sqrt{\{24·63 \div 27\}}$
$= 0·96$ half oz.;

or 2·47 per cent. of the mean yield 38·67 of the sub-plot.

Nitrogen and potash comparisons.

Standard error (mean of 6 plots) $= \sqrt{\{114 \cdot 16 \div 6\}}$
$= 4 \cdot 36$ half oz.;

or 5·64 per cent. of the mean yield 77·34 of the whole plot.

Table XVI. *Summary of results—Mean sampling plant-weight.*

Average yield	Without superphosphate	With superphosphate	Mean	Standard error
Oz. per plant ...	18·78	19·89	19·33	0·48
Per cent.	97·1	102·9	100·0	2·47

		Average yield in oz. per plant S/Amm.			Per cent. S/Amm.		
		0	1½	3	0	1½	3
Potash	0	13·69	20·46	18·12	70·8	105·8	93·7
	1	17·19	20·51	25·55	88·9	106·1	132·1
	2	16·29	17·14	25·05	84·3	88·7	129·6
		Standard error ...		1·09	Standard error ...		5·64

On taking these results in conjunction with the earlier ones, it is apparent that the phosphate effect has been rendered less definite than it was in reality from two causes (*a*) the error of sampling, and (*b*) the variation in plant-number from plot to plot. These causes have acted together and in the same direction; they have reduced the actual difference between the means of the phosphate and no-phosphate sub-plots, and at the same time have increased the standard error of this difference. Apart from this, the sampling method may be held to have been very successful in showing up the main fertiliser effects at only a fraction of the labour and cost necessarily incurred in the complete harvesting and separate weighing of all the plots.

Discussion.

The comparison of errors due to soil differences and experimental errors, with those due to sampling, is best made by the calculation of relative variances $\left(\frac{\text{variance}}{(\text{mean})^2}\right)$ per plot.

Table XVII.

Relative variance $\times 10^4$.

	Rothamsted Whole plots	Rothamsted Half-plots	Woburn
Agricultural yields	88·6	45·1	50·4
Sampling relative variance ...	46·8	93·7	25·6

The sampling variance is unsatisfactorily high as compared with the variance due to other causes, especially in the case of half-plots, where

the fact that adjacent half-plots are considered reduces the variance due to soil differences very considerably. That the high values at Rothamsted are due to some extent to the severe infection with Leaf Roll is borne out by examination of the corresponding figures for Woburn, where, however, the plots were of 1/40th acre. It appears that a 1 in 10 sample is definitely inadequate for plots of 1/90th acre: had the sampling method alone been relied upon, the effect of phosphate would have appeared insignificant. It is somewhat unsatisfactory, too, for plots of 1/45th or 1/40th acre, for even at Woburn the sampling variance was half as great as the agricultural-yield variance. It must in fact be considered fortunate that all the treatment differences for whole plots appeared significant on analysing the sampling-yields in the Rothamsted experiment.

It is easy to estimate from the analyses how many plants it would be necessary to lift from each plot in order to give a sampling-error of, say, 4 per cent. At Rothamsted the number is 102, and at Woburn 56. A sample consisting of this number of plants would raise a standard error per plot from 7 per cent. only to 8 per cent., and the reduced labour of lifting might compensate for the slight loss of accuracy. At Rothamsted it would be necessary to take every third or fourth plant of a plot of 1/40th acre in order to reduce the sampling-error to this extent, while for plots of 1/20th acre every seventh plant would be needed. At Woburn, the error being smaller, every sixth and every twelfth plant respectively would have been sufficient. Little would be gained, then, by the use of a sampling method for small plots. With large plots, of 1/20th acre or more in area, however, the labour costs of lifting and weighing the crop would be very considerably diminished without prejudicing the value of an experiment.

A comparison of the relative variances per plot, derived on the one hand from the agricultural yields and on the other from the sampling yields, should provide an indirect estimate of errors due to the sampling technique. For in the case of the agricultural yields, the plot variance arises from differences in soil-fertility on different plots; from inaccuracies in area measurements and weighings; and from the fact that there is a comparatively small number of plants on a plot. The sampling-yields are subject to all these errors, but to the last in a greater degree, since only one-tenth of the total number of plants is weighed. The differences between the two plot variances, expressed in suitable units, should therefore be an estimate of nine-tenths of the variance due to sampling.

The accompanying table shows the relative variances for whole plots

and for half-plots, arising from factors other than block and treatment differences.

Table XVIII.

Relative variance × 10^4.

	Whole plots	Half-plots
Agricultural yields	88·6	45·1
Sampling yields	178·6	179·4
Difference × 10/9	99·9 }	149·2 }
Sampling variance	46·8 }	93·7 }

The quantities enclosed in brackets should be equal. They appear to differ considerably: the indirect estimate is about double the direct estimate. The reason for the apparent discrepancy is the very high variance of the indirect estimate, based as it is on the difference between two variances. Thus the variance of the difference between two variances v_1 and v_2, based on n degrees of freedom, is given by the formula

$$V_{v_1-v_2} = 2\,(v_1{}^2 + v_2{}^2 - 2r_{v_1 v_2} v_1 v_2)/n,$$

where $r_{v_1 v_2}$ is the correlation between the variances in samples. $r_{v_1 v_2}$ has been shown (4) to be exactly equal to $\rho_{12}{}^2$, where ρ_{12} is the population value of the correlation between the two variates. As the best available estimate of this parameter we may use r_{12}, the value observed in samples. In the present case r_{12} is the "remainder" correlation between the corresponding values of the agricultural and sampling yields.

We have, then,

$$V_{v_1-v_2} = 2\,(v_1{}^2 + v_2{}^2 - 2r_{12}{}^2 v_1 v_2)/n.$$

r_{12} is found to be 0·8233 for whole plots. Using this, and putting

$$v_1 = 88{\cdot}613,$$
$$v_2 = 178{\cdot}554,$$
$$n = 16,$$

we find

$$V_{v_1-v_2} = 2286,$$

or the standard error,

$$S_{v_1-v_2} = \sqrt{2286} = 47{\cdot}8.$$

Now the sampling variance is based on 841 degrees of freedom. Its standard error is therefore

$$\sqrt{\{2 . 46{\cdot}8^2/841\}} = 2{\cdot}28,$$

and the standard error of the difference, 53·1, between the two estimates of sampling variance is

$$\sqrt{\{47{\cdot}8^2 + 2{\cdot}28^2\}} = 47{\cdot}9.$$

Hence the difference, 53·1, barely exceeds its standard error, and is clearly insignificant.

Two interesting points emerge from this calculation. In the first place it is evident that the direct estimate is enormously more accurate than the indirect estimate, their variances being in the ratio of about 1 : 400. If, then, it is required to know the extent to which errors due to the sampling technique contribute to the total errors, it is essential that a direct estimate of the former should be made. This is only possible if at least two sampling-units are taken from each plot. In the present experiment the plots were strictly speaking only sampled in duplicate. At Rothamsted, however, it was found that the sampling-error did not differ significantly from what would have been expected if the units had been distributed wholly at random, *i.e.* if there had been about 17 sampling-units, each a single plant, instead of only 2, each of 8–9 plants.

This leads to the second interesting point—the extent to which the *accuracy* of the estimate of error depends on the number of degrees of freedom on which it is based. The *magnitude* of the sampling-error, and therefore the accuracy of the estimate of yield, is practically unaltered whether it is assumed that 2 or that 17 sampling-units are taken from each plot. But the standard error of the sampling variance is 9·01 in the first case (54 degrees of freedom), and 2·28 in the second (841 degrees of freedom). In this connection it should be noted that some of the advantages of complex sampling-units, but without the disadvantage of reducing the number of degrees of freedom, can be gained by dividing a plot into a small number of parts from each of which equal numbers of simple sampling-units are taken. The sample is the same size as before, and is still fairly representative of the plot, but there being a greater number of independent random locations, the labour of sampling is increased. If the plot is divided into n_1 parts, and n_2 sampling-units are taken from each part, the variance may be analysed thus:

Fraction	Degrees of freedom
Within parts	$n_1(n_2-1)$
Between parts	n_1-1
Total	n_1n_2-1

Hence the degrees of freedom available for estimate of error are reduced only by n_1-1, a number which would usually be not more than 4.

The desirability of adopting this procedure depends upon the relative importance of reducing labour and increasing the accuracy of the estimate of sampling-error: for samples of the same size the magnitude of the error should be much the same in the two cases.

SUMMARY.

(1) The desiderata of a sampling-method are outlined, and the particular case of sampling a large number of potato plots discussed.

(2) An analysis is made of the yields of 54 sub-plots of the Rothamsted Potato Experiment of 1928, both as estimated by a sampling-method and as determined by large-scale lifting.

(3) It is shown that most of the significant results of the experiment are obtained from the sample-yields, but that the higher standard error per plot obscures the effect of superphosphate.

(4) It is concluded that at Rothamsted 102, and at Woburn 56, plants would have to be lifted to give a sampling-error as small as 4 per cent. It would then be profitable only to sample experimental plots of 1/20th acre or more in area.

Finally it is a pleasure to record our indebtedness to Dr R. A. Fisher for much valuable advice and criticism: and to Mr H. J. G. Hines for assistance with the field work.

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EXPERIMENTS IN THE CULTIVATION OF THE SUGAR BEET CROP IN THE WEST MIDLANDS DURING 1928.

A STATISTICAL EXAMINATION OF THE EFFECT OF SPACING.

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As a result of the information obtained in the preliminary experiments in the cultivation of the sugar beet crop during 1927[1], further experiments in cultivation were projected and carried out during the season of 1928. These were designed to throw more light on the question of spacing.

A scheme was accordingly drawn up introducing eight variations, viz. four different distances between the rows, 15″, 18″, 21″ and 24″, and four different spacings between individual plants in the row, viz. 4″, 6″, 8″ and 10″. These in all possible combinations gave 16 plots. To obtain the necessary accuracy it was decided to carry out the experiment in quadruplicate.

The following diagram (Fig. 1) gives the arrangement of the plots.

Each plot consisted of 7 rows, the outer ones of which were discarded at harvest time leaving 5 rows for lifting. The whole scheme covered ¼ acre, the average area per plot was therefore 1/256 acre.

The number of plants per plot varied from 90 on the widest spacing to 225 on the narrowest spacing. The area allotted to each plant, therefore, varied from 60 sq. inches to 240 sq. inches. The number of beet per acre varied from 26,136 on the widest spacing to 104,544 on the narrowest spacing.

This arrangement of plots was devised to fit in with such practical considerations as planting, singling, harvesting, etc. These operations must be carried out in as short a time as possible, or the errors introduced would be considerable.

[1] W. Morley Davies, "Experiments in the Cultivation of the Sugar Beet Crop in the West Midlands during 1927," *Journ. Agric. Sci.* 18, Part IV.

Cultivation, seeding and singling.

After the preliminary ploughing, harrowing, rolling and manuring, the remaining cultural operations were carried out by hand. The seeding, for which a Planet Junior drill was used, was also done by hand. The

				Width of rows
6″	4″	10″	8″	18″
8″	6″	4″	10″	21″
10″	8″	6″	4″	24″
4″	10″	8″	6″	15″
8″	6″	4″	10″	21″
10″	8″	6″	4″	24″
4″	10″	8″	6″	15″
6″	4″	10″	8″	18″
10″	8″	6″	4″	24″
4″	10″	8″	6″	15″
6″	4″	10″	8″	18″
8″	6″	4″	10″	21″
4″	10″	8″	6″	15″
6″	4″	10″	8″	18″
8″	6″	4″	10″	21″
10″	8″	6″	4″	24″

Fig. 1.

plots received an initial dressing of carbonate of lime at the rate of 1 ton per acre. The manuring was as follows:

4 superphosphate
1½ sulphate of ammonia } cwt. per acre at sowing time.
1½ muriate of potash
1 nitrate of soda (after singling—applied June 20th).

The quantity of seed applied was heavier than in the usual practice. It was sown on the flat on May 10th. Precision in laying out the rows was introduced by working to measured distances on a rod. With a few exceptions a very even plant was obtained.

On the 14th, 15th and 16th June, the process of singling took place.

This was effected by placing a rod, marked with appropriate spacings, against the row, and removing all the plants except the one nearest to the mark. In those few cases where gaps occurred, plants were carefully transplanted in from other areas.

HARVESTING AND SAMPLING.

Harvesting operations were commenced on November 6th and completed on November 8th. It must be remembered that the beet crop is much later in reaching maturity in the west than in the east of England, and for that reason the harvesting was later. The weather was settled throughout the harvesting period and the soil reasonably dry.

As mentioned earlier, the two outside rows in each plot, as well as the outside plants round the whole area were discarded.

Fifty beet were drawn, by taking every *n*th beet, as the sample for dirt tare and sugar-content estimations. They were prepared by removing the leaves (*i.e.* to the top of the root) and the crown (*i.e.* down to the last leaf scar) separately. The leaves, crowns and roots were weighed. The remaining beet on the plot were then pulled. From these the tops and crowns were similarly estimated and the beet themselves washed and weighed.

The total weights of dirty and washed beet, the total weights of tops and crowns from each plot were therefore determined, together with dirt tare and sugar content.

In the following tables of yield the figures have been derived from total washed beet per plot, and not by the application of the dirt tare figure from the sample of 50 to the total weight of dirty beet per plot.

RESULTS.

In each block there are 4 rows of varying width. The 15 degrees of freedom yielded by the 16 rows from all the blocks may be partitioned thus:

Blocks ...	...	3
Row-widths	...	3
Remainder	...	9
Total	...	15

As, for practical reasons, it would have been inconvenient to randomise the row-widths and as the hoe-widths have been systematically arranged it is unsafe to partition off the 63 degrees of freedom yielded by the 64 plots and use the 45 degrees of freedom thus made available for the

estimation of the error attaching to our results. Therefore, the hoe-widths have been treated in a manner similar to that used for row-widths and 9 degrees of freedom are then available for the estimation of error as before.

The results for yields of washed beet are shown in Table I A.

Table I A.

	Degrees of freedom	Sum of squares	Mean square	S.D.
Blocks	3	2·16	0·72	—
Row-widths	3	9·17	3·06	—
Remainder	9	4·41	0·49	0·70
Total	15	15·74	1·05	—
Blocks	3	2·16	0·72	—
Hoe-widths	3	1·58	0·53	—
Remainder	9	9·00	1·00	1·00
Total	15	12·74	0·85	—

Comparing the value 3·06 from 3 degrees of freedom with 0·49 from 9 degrees of freedom, the value of z is 0·9148, while the 5 per cent. point from the table of z (R. A. Fisher, 1925, p. 210) is less than 0·7014. The corresponding value of z in the case of hoe-widths is 0·3170, the 5 per cent. point being about 1·089.

Taking 0·70 as the standard error for row-widths and 1·00 as that for hoe-widths, the standard errors of the differences between means of 16 plots are respectively 0·247 and 0·353.

Table I B. *Effect of variation in distances between rows.*

Tons per acre.

	Roots washed beet	Crowns	Tops	Total crop	% sugar	Variation of % sugar content
15″	13·67	1·56	20·58	35·81	17·33	16·72 – 17·93
18″	13·28	1·49	20·29	35·06	17·35	16·92 – 17·85
21″	13·03	1·42	19·92	34·37	17·43	16·81 – 18·19
24″	12·63	1·51	20·12	34·26	17·34	16·78 – 17·85

Standard Error of differences, 0·247.

Significant differences only occur in the case of the yields of roots. Yields of crowns and tops per acre seem independent of spacing between rows. There is a significant difference between the yields on the 15″ and 21″, 15″ and 24″, and 18″ and 24″ spacings. The difference is greatest between the 15″ and 24″ to the extent of about one ton, while it is about two-thirds of a ton in the other two cases. The greatest expectation of yield will be from the narrower spacings.

Table II. *Results from outside centres in the provinces.*

Shropshire. The following figures express the average results from 8 centres:

	Tons per acre washed beet	Sugar %
18″	12·7	17·65
21″	11·5	17·71
24″	9·8	17·43

In the eight instances summarised in Table II one centre only gave a higher result on the 21″ spacing than on the 18″, and in this case the 21″ spacing gave an increase of nearly one ton per acre over the 24″ spacing.

The Agricultural Organiser for Shropshire states that taking both years 1927–1928 into consideration the 18″ spacing gave 6⅓ per cent. increase over the 21″ spacing and 18 per cent. over the 24″ spacing in his experiments.

Table III. *Effect of variation in distance between plants in the rows.*

	Roots washed beet	Crowns	Tops	Total crop	% sugar	Variation of % sugar content
4″	13·43	1·62	21·30	36·35	17·41	16·72–17·90
6″	13·08	1·46	20·38	34·89	17·35	16·81–17·93
8″	13·05	1·41	19·86	34·32	17·33	16·87–17·70
10″	13·05	1·49	19·41	33·95	17·45	16·81–18·19

Standard Error of differences, 0·353.

The differences in the variation in yields of roots were therefore not significant even between the 4″ and 10″ spacings. The differences in the yields of tops were significant between 4″ and 8″, and 4″ and 10″ spacings.

Again there were no significant differences in the yields of the crowns.

It is obvious that there must be an upward limit to the spacing between the plants, and some light was thrown on this in the experiments carried out by the Agricultural Organiser for Staffordshire.

Table IV. *Results from external centres in the province.*

Staffordshire.	Himley	Enville	Gt Bridgford	
Narrow singling 7–8 inches	12·32	13·25	7·05	Tons per acre washed beet
Medium „ 9–10 „	12·68	13·55	7·15	
Wide „ 11–12 „	11·71	12·55	7·05	

These figures corroborate the College results up to 10 inches. Above that there seems to be a dropping off in yield which points to the fact that the maximum spacing allowable is 10 inches.

There were no significant differences in yield per acre and the area allocated to each plant, except that the lowest returns were obtained on the maximum area per plant and the highest on the minimum. There

is no relation either between the area (and therefore the weight) per plant and the sugar content.

Table V. *Effect of area per plant on the yield.*

The following figures summarise the results for variations in area per plant.

Spacing (inches)	Area (sq. in.)	Tons per acre washed beet	Sugar %
24 × 10	240	12·05	17·41
21 × 10	210	13·41	17·59
24 × 8	192	12·37	17·28
18 × 10	180	13·61	17·37
21 × 8	168	12·96	17·26
15 × 10	150	13·12	17·33
18 × 8	144	13·40	17·38
24 × 6	144	12·99	17·36
21 × 6	126	12·81	17·34
15 × 8	120	13·46	17·40
18 × 6	108	12·55	17·32
24 × 4	96	13·11	17·29
15 × 6	90	13·97	17·38
21 × 4	84	12·94	17·54
18 × 4	72	13·55	17·34
15 × 4	60	14·14	17·19

Dirt tare.

As was mentioned early in the paper dirt tare determinations were made on each sample of 50 beet per plot. This was in accordance with the method laid down for the Ministry of Agriculture experiments except that the sample was drawn from the whole plot by taking every *n*th beet rather than from ten half-chain lengths. It is interesting to note that the dirt tare varied from 9–41 lb. per cwt. in spite of the fact that every effort was made to clean the beet uniformly. This wide variation on a soil fairly uniform in texture clearly illustrates the variation possible in commercial consignments.

The higher tares were almost certainly due to fanging, but there was, however, no apparent relation between fanging and spacing. No attempt is made to account for these differences in fanging between one part of the area and another.

Sugar content.

The average sugar content for the whole of the plot area was 17·38 per cent. with a maximum variation of 1·47 per cent. (*i.e.* from 16·72 to 18·19 per cent.). There was no relationship between size of beet and sugar content, a point of considerable interest, but one which is somewhat at variance with the results of some investigators who find that the sugar content decreases with increase in size of the beet.

BOLTERS.

These were counted on August 4th and amounted to 0·4 per cent. of the total beet population. No relation existed between numbers of bolters and spacings. The roots from bolters were included in computing the total yield, but were excluded from the samples of 50.

SUMMARY.

The conclusions of the 1927 experiments in spacing have been tested in greater detail in 1928 and are confirmed.

From the 64-plot scheme containing a series of spacings in quadruplicate the following points emerged:

(1) *Variation of the width between the rows* influenced the yields of sugar beet roots, the expectation of highest yield being on the narrowest spacings. There was a significant increase in yield to be gained by using 15″ or 18″ spacing instead of 21″ or 24″. On the other hand width between the rows did not seem to influence the yield of tops and crowns.

(2) *Variation of the distance between plants in the rows* up to 10″ had no significant effect on the yield of roots. There was some evidence that above that figure a reduction in yield took place. It is interesting to note that in the case of the tops, spacing between the plants influenced the tonnage. There was a significant difference between the yields on the 4″ and 8″, and 4″ and 10″, but not between the 4″ and 6″, 6″ and 8″ or 8″ and 10″ spacings.

(3) There were no significant differences in yield per acre due to *variations in area per plant.* Apparently no substantially greater yields are to be obtained from a plant where the individuals are allocated 72 sq. inches each than where they have 210 sq. inches.

(4) *The dirt tare*, even under conditions where uniformity was sought, varied considerably. On fairly uniform soil fanging is undoubtedly the cause of the variation. Variations from 9–41 lb. per cwt. were obtained. No relation between fanging and spacing was noticeable.

(5) *The sugar content* was a rather more uniform figure than the dirt tare. Its maximum variation was from 16·72 to 18·19 per cent. = 1·47 per cent. The average was 17·38 for the whole area.

(6) *The disposition of bolters* was entirely random. They amounted to 0·4 of the crop.

Conclusions.

The results of two years' experiments point to the desirability of as close spacing as possible between the rows.

Growers need not be as anxious about the spacing in the rows provided that the maximum of 10″ between the plants is not exceeded.

Considerable variations in dirt tare must be expected even under conditions of uniform pulling and cleaning, particularly if the beet are fanged.

Variations of 1·5 per cent. in sugar content are to be expected even on an area as small as $\frac{1}{4}$ acre.

Acknowledgments are made for the help received from colleagues and also from the Agricultural Organisers for Shropshire and Staffordshire for the use of their experimental results. The authors also thank Dr R. A. Fisher, of Rothamsted, for his kind help and criticism.

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BIOCHEMISTRY OF WATERLOGGED SOILS. PART III[1].

DECOMPOSITION OF CARBOHYDRATES WITH SPECIAL REFERENCE TO FORMATION OF ORGANIC ACIDS.

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(With Eight Text-figures.)

In the previous communications (25, 26) the more prominent effects of waterlogging in absence of foreign materials were described. The present paper deals with a study of the changes that attend addition of nitrates and carbohydrates.

Effect of addition of nitrates.

Warington's experiment (29) on percolation of nitrates through waterlogged soil was repeated with a view to determining whether any denitrification occurred under such conditions. Nitrates, after collection, were estimated by the Devarda alloy method (24). It was observed that in absence of plant residues nitrates could be completely recovered by repeated percolation, though the high concentration made the soil sticky and difficult to work with.

Table I.

Nitrates as p.p.m. of nitrogen.

Exp. No.	Soil	Days ...	0	1	3	5	7
I	Rothamsted	...	18·4	17·4	20·1	17·2	20·0
	Indian ...	...	18·4	16·9	15·4	13·6	18·8
II	Rothamsted	...	36·7	35·2	32·5	31·4	35·1
	Indian ...	...	36·7	32·7	26·5	29·1	30·4
III	Rothamsted	...	55·2	53·9	50·8	52·7	56·9
	Indian ...	...	55·2	49·5	47·2	52·6	47·7
IV	Rothamsted	...	73·5	72·1	74·8	72·3	71·9
	Indian ...	...	73·5	68·4	72·4	66·9	65·5
V	Rothamsted	...	92·1	83·8	78·7	81·2	76·1
	Indian ...	...	92·1	79·5	79·8	81·5	79·0

Average diminution ($\bar{x}$) $\div$ standard error $= t^{(6)}$: t for 0–1 day $= 3·5$, which is significant.

In order to determine whether concentration of the nitrate had any effect on its subsequent transformation in the waterlogged soil, potassium nitrate was added in aqueous solution to 100 gm. lots of the same soils as those previously studied, to correspond to different concentrations

[1] Part of thesis accepted by the University of London for the degree of Doctor of Science.

and the mixtures were incubated, the Rothamsted soil at 20° C. and the Indian soil at 35° C., after being waterlogged. Samples were analysed at two-day intervals (Table I).

Though nitrates suffered a slight loss at the end of the 1st day, yet a simultaneous determination of total nitrogen indicated no change, thereby proving that no denitrification had occurred.

EFFECT OF ADDITION OF FERMENTABLE ORGANIC MATTER.

A study of the transformation of organic matter is of fundamental importance and will throw light on the mode of decomposition of (*a*) straw, leaves, etc. which occur in the soil, (*b*) green manures and different other forms of organic fertilisers that are generally allowed to rot in the puddled soil prior to transplanting of paddy, and (*c*) various mineral transformations resulting in increase of plant food that occurs under such conditions. But it is exceedingly difficult to carry out because of (*a*) the complex composition of soil, (*b*) enormous number and variety of soil microflora, each member of which carries out its own characteristic function, and (*c*) want of adequate technique to deal either with the mixed flora or the different products of their metabolism as obtained under waterlogged conditions.

Among the different methods for studying biological activity in the soil the one introduced by Remy (21) and extended by Lohnis (14) is defective because the reactions are allowed to proceed in artificial media which do not represent natural soil conditions. The technique of Withers and Fraps (30) also introduces abnormal conditions because of the use of sterilised soil. The Lipman-Brown (13) and Russell-Hutchinson (23) methods are useful in the study of the release of available plant food, the former from added materials and the latter from unavailable forms present in the soil. In the present study an attempt was made to combine the Russell and Lipman methods with some modifications. Where adequate chemical techniques were lacking new ones were introduced. To avoid complexity due to indeterminate compositions of the added materials the following series of trials were carried out mainly with carbohydrates of well-defined composition.

EFFECT OF ADDED GLUCOSE ON NITRATES.

Glucose was added in solution to correspond to 600, 1200, 1800, 2400 and 3000 parts respectively as carbon per million parts of the soils which were waterlogged as usual (25). Nitrates were determined every 2 days (Table II).

It was observed that even at the end of 24 hours the soils developed a characteristic odour, and were frothy with carbon dioxide, and acid to phenolphthalein even after boiling. At later stages the changes were more pronounced.

Table II.

Nitrates in the Rothamsted and the Indian soils, at the beginning, were 16·1 and 43·8 p.p.m. respectively.

Nitrates as p.p.m. of nitrogen.

Glucose as p.p.m. of carbon	Rothamsted soil				Indian soil			
Days ...	1	3	5	7	1	3	5	7
None	13·4	12·1	10·8	11·7	39·6	41·1	38·6	40·7
600	6·6	2·1	3·5	1·8	16·4	4·7	3·4	1·8
1200	3·0	...	...	...	6·8	2·1	1·6	...
1800	...	...	...	...	3·2	...	...	...

... nil; 2400 and 3000 p.p.m. of carbon—nil throughout.

In order to observe whether addition of glucose caused corresponding losses in total nitrogen a similar series of determinations were made with 30 gm. lots of soils. The results, however, showed that the changes were at no time significant. It should, therefore, be inferred that the rapid disappearance of nitrates was not due to denitrification but to transformation to other forms of nitrogen.

DISSOLVED OXYGEN.

In order to determine whether addition of sugar introduced any change in the oxygen-contents, trials were carried out under the same conditions as in the previous experiments. Since, owing to the constant interchange of oxygen between the soil-sediment and the surface water (25), an increase or decrease in the one would be correspondingly reflected in the other, it was considered sufficient to determine only the amounts of oxygen present in surface water. Rideal and Stewart's modification (22)

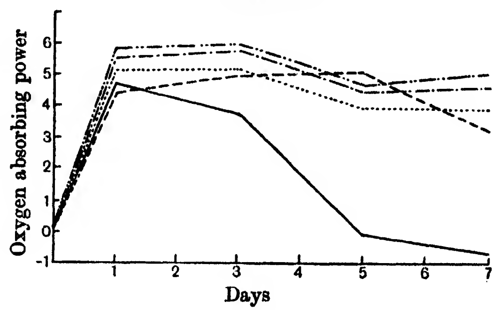
Table III.

Oxygen at the beginning in both soils = 7·3 p.p.m.

Dissolved oxygen as p.p.m.

Glucose as p.p.m. of carbon	Rothamsted soil				Indian soil			
Days ...	1	3	5	7	1	3	5	7
None	7·9	8·7	7·0	8·4	5·8	6·1	5·2	5·6
600	1·6	5·3	5·8	8·0	1·1	2·4	5·3	6·3
1200	1·3	2·8	3·7	4·9	1·4	1·2	0·2	2·4
1800	1·0	1·1	2·1	2·6	0·7	1·0	1·3	1·7
2400	0·5	0·4	1·3	1·7	0·3	0·4	0·8	1·0
3000	0·2	nil	0·4	1·0	nil	0·2	0·6	0·6

of Winkler's method was adopted with correction for dissolved reducing matter. The results (Table III) showed that the oxygen-contents first fell considerably, but soon rose owing to diffusion of oxygen from air. The recovery was rapid when the sugar added was small, but slow when large.



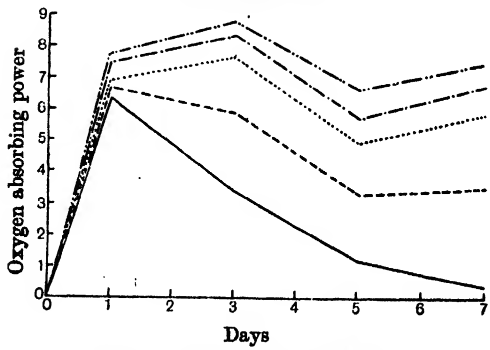


Fig. 1. Oxygen absorbing power.

———— Soil + 600 p.p.m. of carbon. ------ Soil + 1200 p.p.m. of carbon.
·········· Soil + 1800 ,, —·—·— Soil + 2400 ,,
—··—··— Soil + 3000 p.p.m. of carbon.

The addition of sugar led evidently to some internal reaction which caused rapid depletion of oxygen. At later stages oxygen of the air diffused in and restored normal conditions. Since the soils remained exposed to air, oxygen in accordance with the gas laws would have diffused into them only in proportion to their shortage from the normal. "Oxygen absorbing powers" of the soils thus estimated (Fig. 1) measured also, indirectly, the intensity of biological action that was mostly, if not entirely, responsible for the depletion of oxygen.

Carbon dioxide present, dissolved in the surface water, was determined

by absorption in baryta and back-titration against standard acid. The data (Fig. 2), though not absolute measures of the gas produced, were estimates of the concentrations developed at different stages. They are positively correlated to the corresponding figures for oxygen absorbing power and indicate that oxygen was largely utilised for the biological oxidation of the carbohydrate to carbon dioxide.

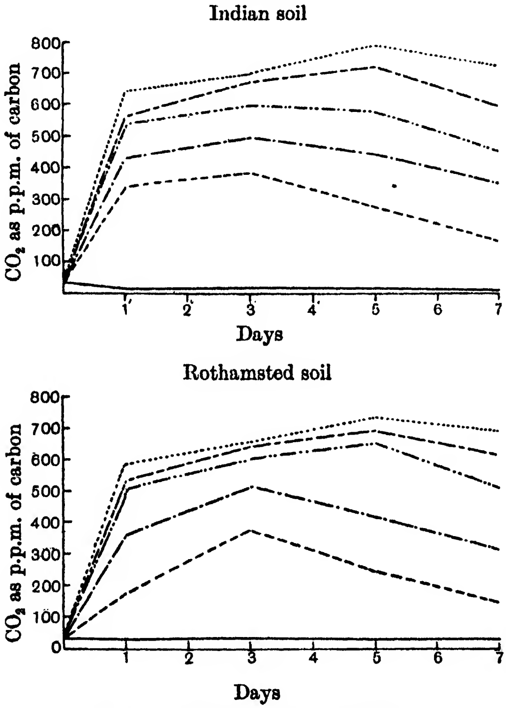


Fig. 2. Dissolved carbon dioxide.

Soil alone.
" + 600 p.p.m. of fermentable carbon.
" +1200 " "
" +1800 " "
" +2400 " "
" +3000 " "

The reaction of aqueous extracts of soils to which sugar had been added, as determined by Gillespie's method (8), indicated a slight initial change (pH 7·5 to 6·5) to acidity. It returned to normal on standing.

Tests were carried out with clarified extracts of soils for the presence of organic acids. Lactic acid was detected by its reduction of permanganate and chromic acid with evolution of acetaldehyde, and by

iodoform, and Fletcher and Hopkins' (7) tests. Acetic and butyric acids were also identified in the liquid by a variety of tests (5).

BACTERIAL NUMBERS.

Bacterial numbers were determined by plating on Thornton's medium (28). It was observed (Fig. 3) that in addition to the great

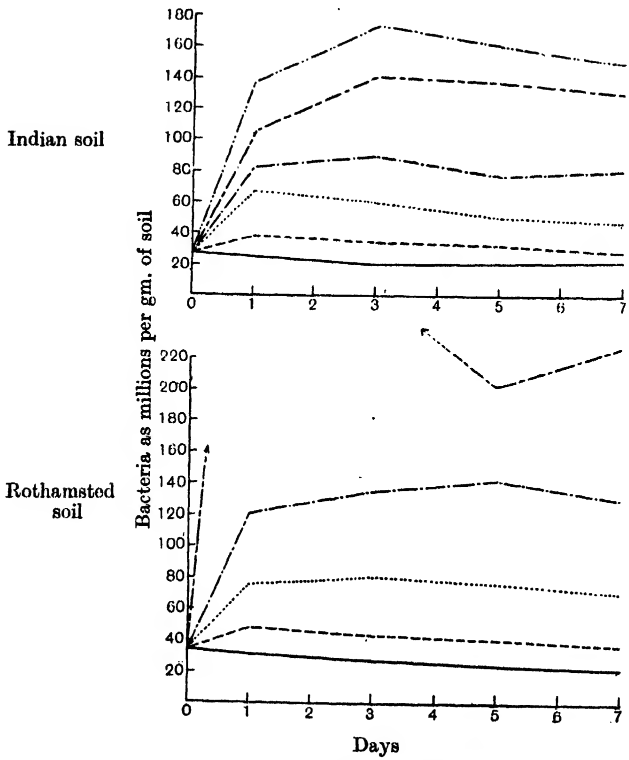


Fig. 3. Bacterial numbers.

———————— Soil alone.
- - - - - - - - ,, +600 p.p.m. of carbon.
.... ,, +1200 ,,
—·—·—·— Soil +1800 p.p.m. of carbon.
— — — — — ,, +2400 ,,
—··—··— ,, +3000 ,,

increase in bacteria, higher concentrations (2400 and 3000 p.p.m.) of the sugar brought out enormous numbers of fungi which rendered counting difficult. The decrease in numbers noticed after the 3rd day might have been due to both shortage of the sugar and to the distinctly visible increase in protozoa.

Correlation between bacterial and oxygen absorption numbers, though positive, was not very close, thereby suggesting that all the bacteria did not depend on oxygen. Many of those responding to the higher concentrations of the sugar might have been either strict or facultative anaerobes. The closer agreement observed between bacterial numbers and CO_2-production lent further support to this view.

ASSIMILATION OF NITRATES BY SOIL ORGANISMS IN PRESENCE OF GLUCOSE.

Soils treated with 600 p.p.m. of the sugar were plated out on Giltay's Agar. After incubation for 7 days at 35° C. the colonies coming out on the plates were examined. The organisms common to all of them, but morphologically different from each other, were inoculated into sterile suspensions of both the soils containing identical amounts of sugar and nearly the same quantities of nitrate by the necessary additions and developed as seed cultures. After they had developed for 24 hours they were transferred into larger quantities of similar soil suspensions. The specimens were incubated for 3 days at 35° C. and then analysed for their nitrate contents (Table IV).

Table IV.

Nitrates as p.p.m. of nitrogen.

	Rothamsted soil		Indian soil		
Designation of the organism used	Present after 3 days	Amount used up	Present after 3 days	Amount used up	Differences between the amounts used up from two soils
A (R)	28·4	42·9	36·6	36·0	+6·9
B	34·1	37·2	41·8	30·8	+6·4
C	37·3	34·0	45·0	27·6	+6·4
D	67·6	3·7	69·2	3·4	[+0·3]
E	23·3	48·0	32·7	39·9	+8·1
A (I)	40·8	30·5	51·3	21·3	+9·2
B	22·7	48·6	30·1	42·5	+6·1
C	69·4	1·9	68·7	3·9	[- 2·0]
D	20·9	50·4	30·3	42·3	+8·1
E	27·2	44·1	35·6	37·0	+7·1

Nitrate originally present in R (Rothamsted soil) = 71·3 p.p.m. and I (Indian soil) = 72·6 p.p.m.

Most of the samples had then turned cloudy with bacterial growth. Some of them had developed characteristic odours. No frothing or gas-production was visible. Except in one case the extracts were all acid to phenolphthalein even after boiling.

The results show that (*a*) nitrate assimilation is common to most soil

organisms, (*b*) where the response of an organism to nitrate is pronounced, the effect of the soil substrate is about the same as instanced by the nearly constant differences (7·3 ± 1·1) observed between the amounts of nitrate assimilated in presence of the two soils, and (*c*) the study of single species of organisms, however prominent, will not be representative: further investigation should, therefore, be carried out with the mixed microflora of the soils.

Relation between concentration of nitrate and the rate of its decomposition in presence of glucose.

100 gm. suspensions of the soils containing 600 p.p.m. of glucose but different proportions of nitrate were incubated, the Rothamsted soil at 20° C. and the Indian soil at 35° C., for 24 hours. They were then analysed for their nitrate-contents and the quantities decomposed by the microflora in each case determined (Table V).

Table V.

Nitrates as p.p.m. of nitrogen.

Rothamsted soil		Indian soil	
Originally present	Decomposed	Originally present	Decomposed
16·1	9·3	43·8	26·7
34·5	19·4	62·2	33·6
52·8	32·1	80·5	41·1
71·3	49·3	99·0	50·8
89·6	65·5	117·3	51·4
108·2	65·1	—	—

The rate of decomposition of nitrate increases with concentration up to a maximum. It is higher for the Rothamsted soil than for the Indian in spite of the lower temperature of incubation and bears evidence to the greater biological activity in the former.

Distribution of carbon and nitrogen in waterlogged soils.

Simultaneous changes in different forms of nitrogen and carbon were studied, using suspensions of soils containing glucose (600 p.p.m.) and nitrate (89·6 p.p.m. in the Rothamsted and 99·0 in the Indian specimens respectively). The soils were waterlogged and incubated under conditions similar to those in the previous series for 48 hours and samples taken for analyses every 12 hours. The determinations were not carried out over a longer period because it was observed that most of the changes took place within 2 days.

Residual sugar at different stages was estimated by a modified Fehling titration method (27): total CO_2 by absorption in baryta, with boiling to drive off that present in the surface water: nitrite by the Davisson method (4): nitrate by the Devarda alloy method (24): ammonia

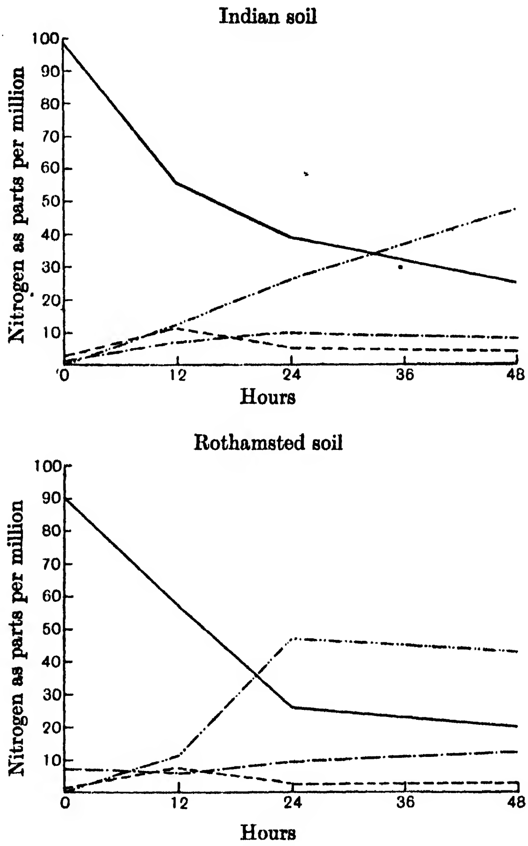


Fig. 4. Distribution of nitrogen.

——— Nitrogen as nitrates. - - - - Nitrogen as nitrites.
—·—·—·— ,, as ammonia. —··—··— ,, assimilated by microflora.

by the McLean and Robinson method (19): total nitrogen by the Gunning-Hubbard method (1): lactic acid by the Buchner and Meisenheimer method (3) after concentrating the neutralised aqueous extract to a small volume: and acetic and butyric acids by steam-distilling at constant volume (27).

CHANGES IN DIFFERENT FORMS OF NITROGEN (Fig. 4).

As was expected nitrates disappeared rapidly: nitrites increased at the same time, suggesting that they were formed mainly from them.

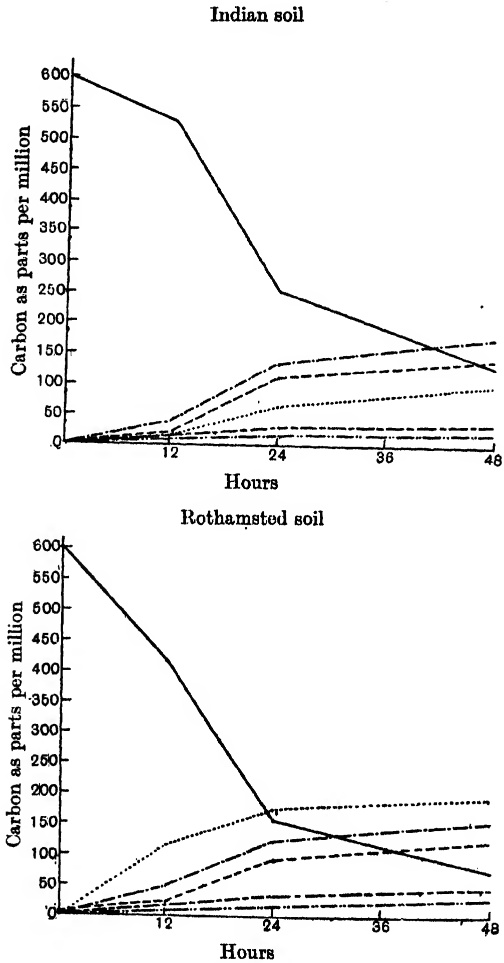


Fig. 5. Distribution of carbon.

———————— Carbohydrate added.	– – – – – – – – CO$_2$ produced.
—·—·—·— Lactic acid formed.	——————— Acetic acid formed.
—··—··— Butyric acid formed.	··········· Carbon taken up by micro-organisms.

Formation of nitrite accords with the observations of Nagaoka (20), Kelly (11) and others. The subsequent decrease in nitrites may have been

due to (*a*) reduction to ammonia, (*b*) direct assimilation by micro-organisms, and (*c*) denitrification. Since total nitrogen remained unaltered throughout the period under observation it should be inferred either that there was no loss by denitrification, or that it was too small to be appreciable. There was significant increase in ammonia in both soils which may have been due to (*a*) the action of the deaminising enzyme present in the soils (26), and (*b*) reduction of nitrates and nitrites by ammonifiers (17).

Nitrogen taken up by micro-organisms or otherwise converted into more complex forms was taken to be approximately the difference between the sums of nitrate, nitrite and ammonia present in the beginning and at the end. The figures showed that the major part of the added nitrogen was thus converted. Since the diminution in the quantities of the nitrates and nitrites present occurred simultaneously with the increased uptake by the micro-organisms and since no other changes were noticeable, it appeared that most of the two soluble forms were thus converted. Since nitrogen of cells of micro-organisms is essentially protein it seems probable that most of the transformed nitrogen was converted into that form.

CHANGES IN DIFFERENT FORMS OF CARBON (Fig. 5).

In both soils the decomposition of the added sugar was extremely rapid. About one-fourth to one-fifth of the total added carbon passed into CO_2. Production of lactic acid accounting for between 25 and 30 per cent. of sugar in both soils was an important change, and may have been due largely to lactic acid bacteria and, to a limited extent, to certain putrefactive and saprophytic organisms like *B. coli* and *B. lactis aerogenes* present in soil. Only small quantities of acetic and butyric acids were formed during the period under investigation. Their molecular proportion was approximately as two of acetic to one of butyric. Quantities of the added carbon converted into complex forms by cells of micro-organisms or otherwise were taken as represented by differences between initial and subsequent amounts present in simpler forms. The figures show that such transformations are greater in Rothamsted than Indian soil. No definite relationship could be found between carbon and nitrogen assimilations.

GENERAL DISTRIBUTION OF THE DECOMPOSED CARBON.

Dividing carbon changes under four heads—(*a*) total converted, (*b*) passing into gas, (*c*) taken up by micro-organisms, (*d*) passing into organic acids—it is evident that the last change is not only unique to the

waterlogged soil, but also the most prominent, involving nearly half the added carbon. The production of acids, particularly lactic, though beginning comparatively slowly, soon proceeded at a rapid rate. Even at the end of 48 hours there was no indication of the reaction weakening.

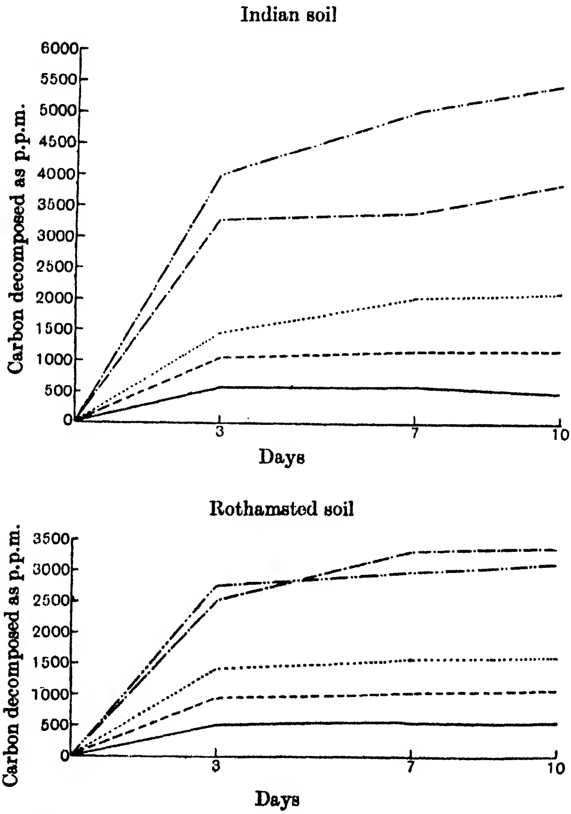


Fig. 6. Effect of concentration on decomposition of fermentable carbon.

———— 600 p.p.m. added carbon. —·—·—· 6000 p.p.m. added carbon.
– – – – – – 1200 „ —··—··— 12,000 „
············ 2400 p.p.m. added carbon.

Since, however, it is known that many of the common bacteria and fungi can not only assimilate organic acids and their salts but also decompose them into simpler compounds (16), the presence of large quantities of organic acids would naturally mean that further biological activity would follow later on. Acid-production would thus be only an intermediate stage in a long series of reactions.

DECOMPOSITION OF ADDED CARBOHYDRATE AT DIFFERENT CONCENTRATIONS.

To specimens of Rothamsted and Indian soils glucose was added from aqueous solution to correspond to 600, 1200, 2400, 6000, and 12,000 p.p.m. of carbon, and after waterlogging incubated for 10 days at 20° C.

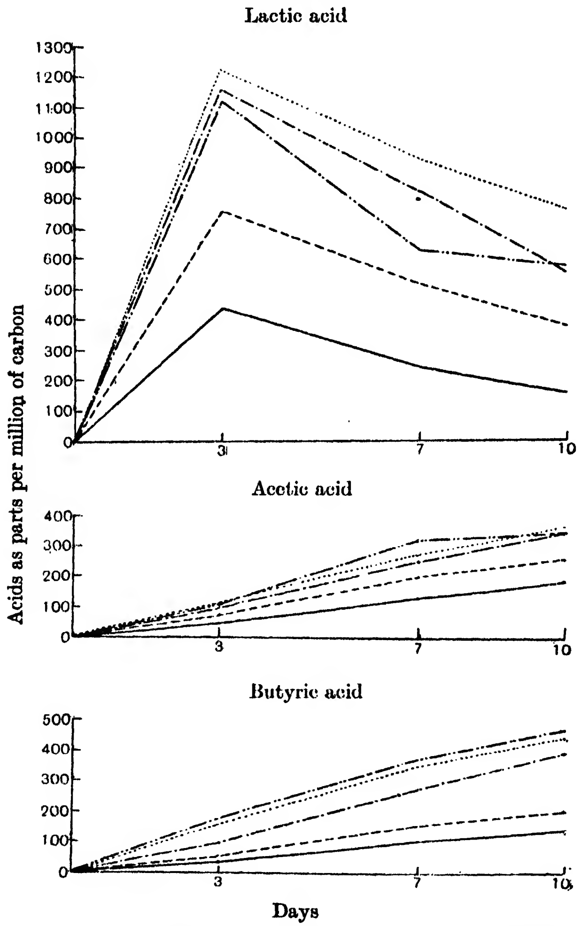


Fig. 7. Formation of lactic, acetic and butyric acids—Rothamsted soil.

————————	2400 p.p.m. fermentable carbon.
– – – – – –	4800 ”
—·—·—·—	7200 ”
············	9600 ”
—··—··—	12,000 ”

and 35° C. respectively. Samples were analysed every 3 days for their sugar-contents (27).

The results (Fig. 6) showed that decomposition of sugar of all concentrations proceeded vigorously up to the end of the 3rd day, after which there was general slackening. This was probably due to shortage of sugar at lower concentrations and accumulation of acids and other products of biological metabolism at higher ones. Total quantities decomposed were, proportionately, much less at higher concentrations than at lower ones.

Production of organic acids at different concentrations of added carbohydrate.

Soils were treated in the same manner as in the previous trial. Lactic acid was estimated by Long's method (15) and acetic and butyric acids by steam-distillation at constant volume (27). Similar types of results were obtained for both soils. Those for Rothamsted soil are plotted in Fig. 7.

At all concentrations of sugar lactic acid was the first product of metabolism and accounted for 30–40 per cent. of the sugar decomposed. After the 3rd day, however, lactic acid tended to diminish, being, evidently, converted into other forms.

Acetic and butyric acids were formed slowly but continuously throughout the period under observation. There was no relation between their quantities and the corresponding amounts of sugar decomposed. Molecular proportions of the two acids were approximately as two of acetic to one of butyric. There was high positive correlation between decrease in lactic and corresponding increase in the other two acids, suggesting that decomposition of the former led, at least partly, to formation of the latter.

Table VI.

Amounts of acids formed as p.p.m. of carbon.

Depth of water	Time in days	Lactic	Acetic	Butyric
Soil just covered	3	912	192	72
	7	516	384	216
	10	264	456	264
2"	3	1164	84	144
	7	432	312	324
	10	336	408	432
4"	3	1272	60	168
	7	684	288	384
	10	432	432	456

RELATION BETWEEN DEPTH OF WATER AND PRODUCTION OF ORGANIC ACIDS.

Specimens of Rothamsted soil containing 6000 p.p.m. of glucose were suspended under different depths of water and the acids formed at the end of 3, 7 and 10 days estimated (Table VI).

Greater depths led to larger production of lactic and butyric acids, suggesting that their formation did not depend on free supply of air. Diminished production of acetic acid under similar conditions suggested the reverse.

EFFECT OF ADDITION OF CALCIUM CARBONATE ON PRODUCTION OF ACIDS.

To 30 gm. portions of the soils containing 6000 p.p.m. of glucose, 2 gm. of calcium carbonate were added before waterlogging. The acids formed were estimated at the end of 3, 7 and 10 days (Table VII).

Table VII.

Amounts of acids as p.p.m. of carbon.

Treatment	Time in days	Rothamsted soil			Indian soil		
		Lactic	Acetic	Butyric	Lactic	Acetic	Butyric
Without $CaCO_3$	3	1164	100	95	1224	118	139
	7	816	252	276	494	384	516
	10	552	348	396	256	432	624
With $CaCO_3$	3	1536	118	121	1788	152	166
	7	745	334	451	991	387	494
	10	374	392	506	716	451	577

Addition of calcium carbonate led to distinct increase in lactic acid: subsequent decomposition of the acid was also rapid, particularly in Rothamsted soil.

The effect of carbonate on the production of the other acids was not pronounced.

PRODUCTION OF ORGANIC ACIDS DURING AN EXTENDED PERIOD OF OBSERVATION.

Specimens of the Rothamsted soil containing 6000 p.p.m. of glucose were analysed for their acid-contents at intervals during a period of 30 days.

It was observed (Fig. 8) that lactic acid decomposed actively and fairly uniformly. Acetic and butyric acids, after increasing till about the 15th day, decomposed appreciably at later stages. The results support

the theory, already suggested, that the acids would, on standing, be attacked by micro-organisms and converted into other forms.

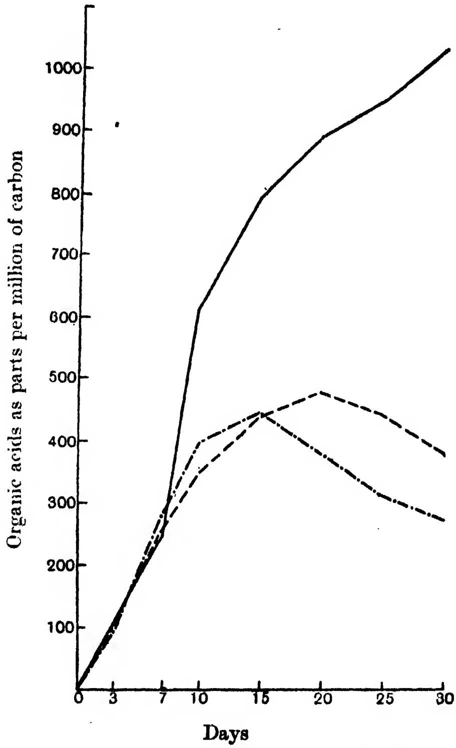


Fig. 8. Changes in organic acids over a long period.

——————— Lactic acid decomposed. – – – – – – Acetic acid produced.
—·—·—·— Butyric acid produced.

Production of lactic acid under aerobic and anaerobic conditions.

20 gm. lots of Rothamsted soil were waterlogged with 4000 p.p.m. of glucose. In one set the mixture was allowed to stand in shallow, glass-covered trays. In the other, after being made up with air-free water, it was placed in narrow-necked flasks connected with U-tubes containing alkaline pyrogallol. Both sets were maintained at the ordinary temperature (20–25° C.). Lactic acid present on successive days was estimated (Table VIII).

Table VIII.

Lactic acid formed as p.p.m. of carbon.

Time in days	Aerobic: Control soil and water alone	Aerobic: With the sugar	Anaerobic: Control soil and water alone	Anaerobic: With the sugar
1	27	378	45	567
2	54	907	63	855
3	45	1134	54	1071

Production of lactic acid occurred under both aerobic and anaerobic conditions and to about the same extent.

FORMATION OF LACTIC ACID IN ABSENCE OF LIVING ORGANISMS.

20 gm. lots of Rothamsted soil were weighed out into dishes with glass covers and allowed to remain soaked in toluene at the ordinary temperature for 48 hours, after which 50 c.c. of distilled water and 20 c.c. portions of 1 per cent. solutions of glucose, sucrose and lactose respectively were added to them. The mixtures were allowed to stand for 3 days. Lactic acid in this and the subsequent series was estimated by the author's modification (27) of the chromic acid method (Table IX).

Table IX.

Lactic acid formed as p.p.m. of carbon.

Time in days	Control soil alone	Glucose	Sucrose	Lactose
1	27	108	189	72
2	54	207	243	189
3	54	234	297	216

It is probable that part of the lactic acid formed during waterlogging was enzymic in origin. The major part, however, was evidently formed by the activity of living organisms.

MECHANISM OF PRODUCTION OF LACTIC ACID.

Since the acid was formed under both aerobic and anaerobic conditions, its production did not involve the utilisation of atmospheric oxygen. Nor is it probable that any biological reduction of the sugar was involved since the acid is a more highly oxygenated product than the sugar. The chemical mechanism was probably either hydrolysis or direct splitting of the carbohydrate. Though Hoppe-Seyler (10) and later Kiliani (12) showed that lactic acid can be formed by the hydrolysis of certain carbohydrates, yet the conditions of their experiments bear no analogy to the production of the acid in the waterlogged soil. Since it

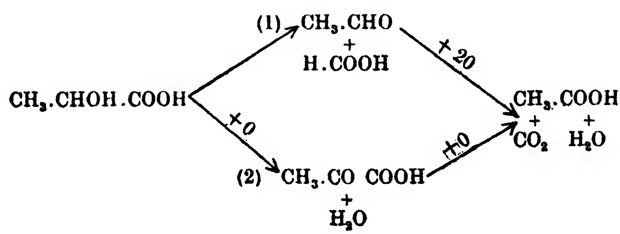
was also observed on trial that the acid was not formed by acid-hydrolysis of the sugars even at 100° C. it may be inferred that hydrolysis was not the mechanism of its production. Biological production of lactic acid was therefore probably one of fission of the carbohydrate molecule which did not bear any relation to the reactions brought about by common chemical reagents. Formation of the acid from glucose would then be represented in outline by $C_6H_{12}O_6 \longrightarrow 2C_3H_6O_3$. The primary change involved in its production would therefore appear to be intramolecular rearrangement of the sugar.

Production of acetic and butyric acids from lactic acid.

In order to test whether any of the fatty acids were formed directly from lactic acid, quantities of the latter corresponding to 1200 p.p.m. of carbon were added to the Rothamsted soil and waterlogged under aerobic and anaerobic conditions. The former was effected by gentle bubbling of air through the suspension and the latter by using air-free water and connecting the flasks to **U**-tubes containing alkaline pyrogallol. The fatty acids were identified and estimated at intervals of 24 hours (Table X).

The extent of decomposition of lactic acid was about the same under aerobic and anaerobic conditions. Under anaerobic conditions the greater part of it passed into fatty acids, while only less than half did so under the aerobic.

The quantities of acetic acid produced under aerobic conditions were uniformly greater than those produced anaerobically. In the former it was formed almost to the entire exclusion of butyric acid and appeared to have been mainly due to oxidation of lactic acid which might have been through the stages of (1) acetaldehyde or (2) pyruvic acid. Both types of reactions might also have taken place simultaneously.



Qualitative analyses made from time to time showed only the presence of pyruvic acid, as indicated by the nitroprusside, β-naphthol and phenylhydrazine reactions. Positive reactions for the aldehyde

could at no time be obtained. As shown by Maze and Ruot (18) it is probable that certain fungi brought about the oxidation of lactic into pyruvic acid.

Table X.

Acids as p.p.m. of carbon.

Time in days	Aerobic Lactic	Aerobic Acetic	Aerobic Butyric	Anaerobic Lactic	Anaerobic Acetic	Anaerobic Butyric
1	984	96	24	996	48	132
2	816	264	48	744	168	276
3	552	216	36	624	192	336

Attempts at reproducing the chemical oxidation of lactic acid at concentrations similar to those obtained in the waterlogged soil to pyruvic acid by the Aristoff method (2) resulted only in the formation of acetaldehyde as the intermediate product. The biological formation of pyruvic acid which proceeds under nearly neutral conditions (pH 6·5) has evidently a different mechanism.

Production of the fatty acids seems to proceed along different lines under anaerobic conditions. Neither pyruvic acid nor aldehyde could be detected at any time.

The molecular proportion under fully aerobic conditions was as 12 of acetic to 1 of butyric and, under anaerobic, approximately as 1 : 1. Since in the waterlogged soil it was 2 : 1 it should be inferred that the conditions therein were intermediate between the above two.

ORGANISMS RESPONSIBLE FOR THE DIRECT ASSIMILATION OF LACTIC ACID.

To isolate the organisms directly assimilating lactic acid the soils were plated out on a medium containing lactic acid (0·2 per cent.), soil extract (sp. gr. 1·002), agar (2·0 per cent.) and a few drops of brom-cresol-purple indicator. After incubation for 10 days at the ordinary temperature the organisms turning the medium purple (*i.e.* destroying the acid and thereby rendering the medium neutral) were examined.

The plates from the Rothamsted soil contained a large number of colonies of a micrococcus which produced the purple within a week. There were also rapidly growing colonies of a *Penicillium* which did not produce the colour till the 10th day. Plates from the Indian soil also contained the *Penicillium*, but the coccoid forms were not to be found. On inoculating the two organisms into lactic acid (45 milligrams) diluted with soil extract (100 c.c., sp. gr. 1·002) it was observed that the acid was rapidly assimilated (Table XI).

Table XI.

Lactic acid assimilated in mg.

Organism	3 days	7 days
Micrococcus	9·7	14·1
Penicillium	12·0	28·7

No acetic or butyric acid was formed at the same time. Qualitative examination showed that the mucilaginous development of the coccus was a carbohydrate resembling glycogen in iodine reaction.

Production of organic acids from different carbohydrates.

Specimens of the Rothamsted soil were treated with (1) glycerol, (2) xylose, (3) arabinose, (4) laevulose, (5) mannitol, (6) sucrose, (7) maltose, (8) lactose, (9) starch, (10) maltodextrin and (11) cellulose (cotton-wool), each in quantities corresponding to 6000 p.p.m. of carbon, waterlogged at the laboratory temperature (20–25° C.). The acids formed at different stages were estimated (Table XII).

Table XII.

Organic acids as p.p.m. of carbon.

No.	Time in days	Lactic	Acetic	Butyric
1	3	67	35	82
	7	112	58	61
2	3	314	46	76
	7	88	118	62
3	3	1126	200	204
	7	272	498	292
4	3	978	272	331
	7	421	326	484
5	3	1187	18	278
	7	321	86	543
	10	119	401	446
6	3	679	126	320
	7	214	333	513
7	3	1688	540	291
	7	796	762	161
8	3	892	306	112
	7	264	416	268
9	3	382	102	101
	7	204	128	62
10	3	436	115	251
	7	196	85	234

Minute quantities of the acids were also formed from cellulose. Formation of the acids was generally accompanied by production of carbon dioxide. The quantities of acetic and butyric acids did not bear

any relation to each other or to decomposed lactic acid. Though the same acids were formed in every case their modes of formation appeared to vary.

SUMMARY.

(1) In absence of decomposing organic matter addition of nitrate led to no loss of nitrogen.

(2) On addition of small quantities of fermentable matter such as glucose there was (*a*) rapid depletion of nitrates and oxygen, but no denitrification, and (*b*) increase in acidity, carbon dioxide and bacteria. The greater part of the soluble nitrogen was assimilated by micro-organisms or otherwise converted and the greater part of the added carbohydrate was transformed into lactic, acetic and butyric acids.

(3) The organic acids were formed from a variety of carbohydrates. Lactic acid was the first to be observed and appeared to be formed mainly by direct splitting of the sugar. It decomposed readily, forming acetic and butyric acids. Some acetic acid was formed by direct oxidation of lactic acid, with pyruvic acid as the intermediate product. All the acids were, on standing, converted into other forms by micro-organisms.

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DETERMINATION OF SOLUBLE CARBOHYDRATES, LACTIC ACID AND VOLATILE FATTY ACIDS IN SOILS AND BIOLOGICAL MEDIA[1].

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(With One Text-figure.)

In the course of a study of the decomposition of carbohydrates in water-logged soils (5) special techniques for estimating different products were adopted owing to the ones then available being neither accurate nor directly suited to soil conditions. Since these methods are also applicable to the study of a variety of other problems they have been described in the present paper.

Soluble carbohydrates.

The soil was treated with about five times its weight of distilled water saturated with thymol and one-fifth of its weight of alumina cream (6). The mixture was shaken vigorously for 5 minutes in a stoppered bottle and filtered on the Buchner with liberal washing. The filtrate was concentrated to 20–30 c.c. by direct boiling if it contained less than 0·5 gm. sugar, and, *in vacuo*, if more. Humic matter was removed by treatment with basic lead acetate (1) and the excess of the latter removed with sodium sulphate.

When the sugar was above 1 per cent. of the soil, the determination was made by direct titration against Soxhlet's Fehling solution (1) using methylene blue as internal indicator (3). Corrections were made after trials, for (*a*) sugar retained by soil and basic lead acetate and (*b*) Fehling reducing matter of soil. Experiments with representative English and Indian soils showed that 97–99 per cent. of added dextrose was recovered and that the reducing matter corresponded to 0·2–0·6 c.c. Fehling solution for every 50 gm. of soil. The errors were constant when identical quantities of soil and clarifying agent were used.

For smaller amounts of sugar, the extract was concentrated to 30-40 c.c., mixed with 50 c.c. of double-strength Fehling solution, and made up to 100 c.c. in a standard flask. Aliquot parts of the mixture were titrated against standard sugar solution. The total amount of sugar originally present in soil was calculated as in back-titration methods,

[1] Part of thesis accepted by the University of London for the degree of Doctor of Science.

after applying the corrections mentioned already. Table I compares data thus obtained for solutions of pure dextrose with those by direct titration.

Table I.

	Direct titration		Back titration	
Vol. of dextrose solution (approx. 1 %) taken	Vol. required for 10 c.c. standard Fehling's	Weight of dextrose as mg. per 100 c.c.	No. of c.c. standard sugar required for the titration of 10 c.c. Fehling's sugar mixture	Weight of dextrose as mg. per 100 c.c.
50 c.c.	10·2 c.c.	490·2	—	—
40	12·8	390·6	2·1	389·3
30	16·8	292·6*	4·1	293·2
20	25·6	194·8*	6·2	192·3
15	34·7	145·1*	7·2	144·2

* From Lane and Eynon's tables. The standard error of difference between the two methods = ±0·43 per cent.

Trials carried out by adding known quantities of dextrose to a Rothamsted soil and determining them by back titration, showed that the method was quite accurate (Table II).

Table II.

[Volumes were made up to 100 c.c. in each case. Reducing matter from 50 gm. of soil = 0·6 c.c. of standard sugar solution.]

Standard error of recovery = ±0·76 per cent.

Vol. of 1 % dextrose added to 50 gm. soil	Vol. of the Fehling's sugar mixture taken for final titration	Vol. of the standard 0·5 % dextrose solution required	Recovery of added sugar
30 c.c.	10 c.c.	4·2 c.c.	97·1 %
10	20	16·3	96·6
5	25	22·9	96·4
3	50	47·7	98·1

Disaccharides.

Approximately 1 per cent. solutions (100 c.c.) of disaccharides were added to a Rothamsted soil (50 gm.) and determined by the direct-titration method (Table III). Sucrose was inverted by acid hydrolysis prior to titration.

Table III.

Sugar	*Control.* Vol. of the standard sugar solution required for 100 c.c. Fehling's	Vol. of extract required to reduce 10 c.c. Fehling's	Vol. of sugar solution equivalent to reducing matter from 50 gm. soil	Percentage recovery of added sugar
Maltose	13·4 c.c.	13·9 c.c.	0·9 c.c.	96·3
Lactose	12·8	13·0	0·8	98·0
Sucrose	9·7	9·9	0·5	97·7

Similar trials were carried out by the back-titration method using smaller quantities (5 c.c. to 50 gm. of soil) of the same sugars. The accuracy obtained was 97 ± 0·7 per cent.

MEASURING MICROBIAL ACTIVITY.

To test the applicability of the back-titration technique, specimens of soils from I, Barn Field, Plot O, Rothamsted; II, Central Farm, Coimbatore; III, Karjat, Bombay, and IV, Punjab, were each treated with 1 per cent. of their weights of dextrose and the decomposition of the sugar at 20° C. and 15 per cent. moisture studied at intervals. The data obtained indicate the microbiological activities in the soils at different stages.

Table IV.

Soil no.	Sugar present as percentages			
	8 hours	24 hours	48 hours	72 hours
I	84	49·6	11·7	1·4
II	96·4	76·7	49·6	20·4
III	98·5	86·9	65·0	56·5
IV	92·7	62·3	44·2	10·1

LACTIC ACID.

This was determined by a modification of Paessler's method (4), since preliminary trials with minute quantities of lactic acid, such as were formed in the water-logged soil, showed (Table V) that complete oxidation of lactic acid to acetic acid did not take place because of the escape of appreciable quantities of the intermediate product, acetaldehyde, into air by bumping during refluxing.

Table V.

Lactic acid taken = 0·03636 gm. 5 c.c. of 1·005 $N/10$ dichromate + 2 c.c. 1 : 1 sulphuric acid was used for oxidation. Volume made up to 250 c.c. after refluxing.

Water added	Vol. of 1·036 $N/50$ $Na_2S_2O_3$ solution required for 25 c.c. of final mixture	Lactic acid found
None	18·70 c.c.	0·0247 gm.
50 c.c.	16·95	0·0329
100	17·25	0·0315
150	17·85	0·0287

Experiments carried out at lower temperatures in small glass-covered dishes showed (Fig. 1) that at 35° C. the oxidation of lactic acid to acetic acid proceeded to completion in about 48 hours without any special attention being required. At a lower temperature the reaction was slow whilst at higher ones there was appreciable loss of aldehyde by volatilisation.

To test the applicability of the method, 20 gm. lots of a soil from Kaliganj, Bengal, were treated with glucose in quantities increasing up to 0·2 gm. and the same amount of lactic acid as used in the previous trial. After vigorous shaking with water and filtration, the extracts were rendered slightly alkaline, concentrated by boiling to about 20 c.c., treated with cupric hydroxide(7) and the filtrate, after treatment with

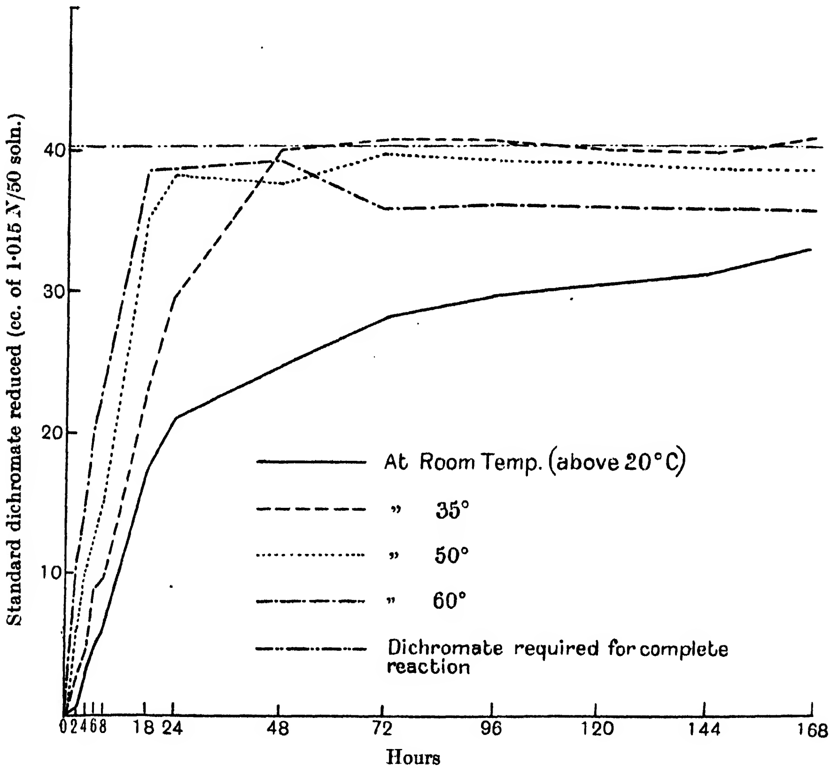

Fig. 1. Quantities of dichromate reacting with lactic acid at different intervals.

dichromate and acid, incubated at 35° C. for two days. Unused dichromate was titrated against thiosulphate iodimetrically.

The figures obtained were identical with those for 35° C. in Fig. 1 showing that (*a*) lactic acid was completely leached out from the soil with water, (*b*) cupric hydroxide removed all the reducing sugars, and (*c*) dichromate oxidation at 35° C. provided an easy and accurate method for determining lactic acid.

VOLATILE FATTY ACIDS.

These were estimated by a modification of Dyer's method (2) of steam-distillation at constant volume. To test the applicability of the method to such low concentrations as were obtained in the water-logged soils, distillations were carried out with dilute solutions of acetic and butyric acids and the constants for different fractions calculated (Tables VI and VII).

Table VI. *Distilling constants of acetic acid.*

Vol. of acid taken	1st 100 c.c.	2nd 100 c.c.	3rd 100 c.c.	4th 100 c.c.	5th 100 c.c.	6th 100 c.c.
5 c.c.	30·6	30·3	30·1	28·5	26·5	22·2
10	30·9	30·5	30·3	29·9	27·9	26·2
15	31·0	30·5	30·4	30·1	29·6	28·5
25	30·7	30·6	30·5	30·3	30·1	29·7
Means with standard errors	30·8 ± 0·2	30·5 ± 0·1	30·3 ± 0·2	29·7 ± 0·7	28·5 ± 1·4	26·7 ± 2·9

Table VII. *Distilling constants of butyric acid.*

Vol. of acid taken	1st 100 c.c.	2nd 100 c.c.	3rd 100 c.c.	4th 100 c.c.
5 c.c.	70·0	69·1	66·7	—
10	69·7	69·4	68·3	63·2
15	70·1	69·7	68·2	64·3
25	69·8	69·7	69·6	66·7
Means with standard errors	69·9 ± 0·2	69·5 ± 0·35	68·2 ± 0·3	64·7 ± 1·5

The fractions distilling over became unreliable beyond the third 100 c.c. Average distilling constants at the lower concentrations for the first two 100 c.c. were 30·6 ± 0·2 and 69·7 ± 0·3 for acetic and butyric acids respectively.

Titration figures for mixtures of acetic and butyric acids (Table VIII) agreed with the distilling constants only for the first two 100 c.c. portions.

Table VIII.

		Alkali (1·042 $N/50$) required					
		1st 100 c.c.		2nd 100 c.c.		3rd 100 c.c.	
Vol. of acetic acid taken	Vol. of butyric acid taken	Calculated c.c.	Found c.c.	Calculated c.c.	Found c.c.	Calculated c.c.	Found c.c.
5 c.c.	25 c.c.	125·0	124·8	42·7	42·2	16·3	14·7
10	15	92·3	92·6	37·7	37·8	18·1	17·3
15	10	82·2	93·0	39·5	38·8	22·0	21·1
25	5	84·5	84·6	49·8	49·3	31·9	31·4
Deviations from those expected		±0·5 %		±1·2 %		±5·8 %	

Table IX shows that the presence of minute quantities of lactic and

sulphuric acids did not appreciably affect the rates of distillation of the fatty acids:

Table IX.

Mixtures containing 10 c.c. each of approximately 1 per cent. solutions of acetic and butyric acids were taken. The expected titration figures for the 1st and 2nd 100 c.c. were 69·8 and 30·9 c.c. respectively of 1·042 $N/50$ alkali.

Alkali (1·042 $N/50$) required.

Vol. of 0·5 % lactic acid added	1st 100 c.c.	2nd 100 c.c.	Vol. of 1 : 8 H_2SO_4 added	1st 100 c.c.	2nd 100 c.c.
5 c.c.	70·1 c.c.	31·1 c.c.	0·5 c.c.	69·8 c.c.	30·8 c.c.
10	70·0	30·4	1·0	70·2	30·9
15	69·7	30·7	2·0	70·3	31·0
25	70·2	31·0	3·0	70·5	31·3
Deviations from figures expected	±0·4 %	±0·9 %	—	±0·7 %	±0·6 %

Extraction and determination.

To 25 gm. lots of six specimens of soils acetic and butyric acids were added. After being treated with about 200 c.c. of distilled water, the suspensions were shaken and filtered with liberal washing. The filtrate after neutralisation was concentrated to about 100 c.c., just acidified with 1 : 8 H_2SO_4 and steam distilled. After titrating the fractions the actual amounts of acids extracted were calculated thus: Let x represent the amount of acetic acid and y that of butyric acid present in the extracts, expressed in terms of the alkali required for neutralisation. Let d_1 and d_2 be the distilling constants for acetic and butyric acids and t_1 and t_2 the titration figures for the first and the second 100 c.c. lots respectively. Then

$$x\frac{d_1}{100} + y\frac{d_2}{100} = t_1 \qquad \text{......(1)},$$

$$x\frac{d_1(100-d_1)}{100^2} + y\frac{d_2(100-d_2)}{100^2} = t_2 \qquad \text{......(2)},$$

give the values in Table X.

Table X.

Acetic and butyric acids actually added equalled 81·0 and 64·6 c.c. respectively of 1·042 $N/50$ alkali: d_1 and d_2 were taken to be 30·6 and 69·7 respectively.

Alkali (1·042 $N/50$) required for neutralisation

Name of soil	Acetic acid recovered c.c.	Error %	Butyric acid recovered c.c.	Error %
Central Farm, Coimbatore—garden soil ...	82·5	+1·9	63·8	−1·2
Rothamsted, Barn Field—dunged plot ...	80·3	−0·9	64·6	0·0
Anakapalli, Madras—paddy	82·3	+1·6	64·4	+0·3
Yessgaon, Bombay	79·6	−1·7	65·3	+1·1
Punjâb—wheat	80·7	−0·4	64·7	+0·2
Mandalay, Burma—paddy	80·5	−0·6	65·4	+1·2
Probable error of a single determination ...	±0·9 %		±0·6 %	

It has to be inferred that (*a*) the extraction by leaching was quantitative and (*b*) the technique adopted led to accurate determination of the acids.

Effect of mass of soil and time of standing.

Further trials (Table XI) showed that the results were not affected by variations in the amount of soil or the time of standing in contact with sterile soil.

Table XI.

Paddy soil from Anakapalli, Madras, was used. Acetic and butyric acids added equalled 73·6 and 107·4 c.c. of 1·042 $N/50$ alkali respectively.

Acids recovered in terms of 1·042 $N/50$ alkali.

Weight of soil	Acetic acid	Butyric acid	Time of standing with 25 gm. of soil	Acetic acid	Butyric acid
15 gm.	73·2 c.c.	108·0 c.c.	4 hours	72·8 c.c.	106·8 c.c.
50	74·0	107·2	8	73·8	106·2
75	73·8	107·0	24	72·6	106·6
100	74·2	106·8	—	—	—
Probable error of a single determination	±0·4 %	±0·3 %		±0·7 %	±0·6 %

Distilling constants for propionic and isobutyric acids at low concentrations were found to be 52·5 ± 0·2 and 79·6 ± 0·3 respectively.

Soils are but complex biological media and the foregoing methods which were applicable to a variety of soils can, with greater facility, be extended to study changes in other types of biological media as well.

SUMMARY.

Methods for extraction, concentration and determination of minute quantities of soluble carbohydrates, lactic acid and volatile fatty acids have been described. Different factors affecting the accuracy of the determinations have been studied and corrections, where necessary, have been suggested.

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THE VALUE OF DRIED SUGAR-BEET PULP AND MOLASSES-SUGAR BEET PULP IN THE NUTRITION OF SWINE.

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Introduction.

The results of an investigation into the utilisation of sugar-beet pulp by *ruminant* animals were brought forward in a recent publication (1). It was demonstrated that sugar-beet pulp is highly digestible when consumed by ruminants. In respect of the digestibility of its N-free extractives and total organic matter, it compares very satisfactorily with maize meal. The process of drying the wet beet pulp in the factory does not depress its digestibility. Further, from the standpoint of digestibility, it is immaterial whether sugar-beet pulp is included in the rations of ruminants in the dry or the soaked condition. When, however, liberal allowances of the dried product are being fed to animals, it is desirable that the food should be well softened in water before feeding. This procedure ensures a higher availability of the digestible nutrients for productive purposes in the animal and also averts risk of choking trouble which sometimes arises, especially with sheep and lambs, during consumption of the dried beet pulp.

It was also shown that the fibrous constituent of sugar-beet pulp is very little inferior in respect of digestibility to the N-free extractives, a result which justified the conclusion that the fibre in this feeding stuff is present almost wholly in the form of simple cellulose, unmixed with any significant amount of the indigestible lignocellulose. The digestion coefficient of the protein constituent was relatively low, and it would appear that the inclusion of sugar-beet pulp in the ration may have the effect of slightly depressing the extent to which the animal is able to utilise the protein in its food.

The final conclusion was drawn that dried sugar-beet pulp must be regarded as a carbohydrate concentrate, 1 lb. of which is able to replace

0·8 lb. of maize or 0·9 lb. of barley in the productive part of the rations of ruminant animals. From the standpoint of price per unit or per lb. of starch equivalent, dried sugar-beet pulp, at present prices, is a cheap source of digestible carbohydrate in comparison with either maize meal or barley meal.

Scope of present investigation.

The question naturally arises as to the suitability of sugar-beet pulp for inclusion in the rations of pigs. In the communication already referred to (1), it was pointed out that almost four-fifths of the dry matter of this feeding stuff (namely, the crude fibre together with the N-free extractives, which are composed mainly of pectose) was digested not by the normal enzymic processes, but by the agency of bacteria. For this reason, it appeared probable that the digestion of sugar-beet pulp in the pig would be much less efficient than in the ruminant animal. It was ascertained, by means of a questionnaire which was sent to a number of well-known farmers, that stockfeeders in this country had but little experience of feeding sugar-beet pulp to pigs, but that where this had been tried the results had not been very encouraging (2).

The experiments to be described in the present communication were carried out with the object of securing reliable information about the value of sugar-beet pulp for pigs. Two separate lines of enquiry were followed: (1) digestion trials with pigs on dried sugar-beet pulp and molasses-sugar beet pulp; (2) large-scale feeding trials with pigs under ordinary farm conditions. It was thought that the combined evidence of this dual enquiry should enable trustworthy conclusions to be drawn.

I. Digestion trials.

For the purpose of the digestion trials, two pure-bred Large White hogs, weighing 190 and 191 lb. respectively at the commencement of the experiment, were employed. In the first period of feeding, the digestibility of a ration composed of fish meal, middlings, maize meal and molasses-beet pulp was determined. The second period was devoted to measuring the digestibility of the basal ration of fish meal, middlings and maize meal, whilst in the final period the molasses-sugar beet pulp of the first experimental ration was replaced by dried sugar-beet pulp. The details of the experimental rations are shown in Table I.

The rations were soaked overnight in water before being fed to the pigs, the amounts of water required being 6000 c.c. in Period I, 4000 c.c. in Period II and 5000 c.c. in Period III. In every period the food was given

in four equal meals during the day. Some difficulty was experienced in bringing the animals on to the molasses-beet pulp diet owing to the bulkiness of the ration after soaking in water. The food in this period was soaked overnight in 4000 c.c. water, this volume being absorbed completely. A further 2000 c.c. water was added on the morning of feeding in order to render the mixture more fluid and thus to facilitate its consumption by the pigs. Although the animals in the early stages did not take readily to the ration, this diffidence disappeared after several days and clean consumption was secured without difficulty during the actual period of the digestion trial. Attempts to exceed the allowance of 500 gm. of molasses-sugar beet pulp showed that any such increase was accompanied by a decrease in the animal's zest for the mixture.

Table I. *Details of digestion rations.*

	Period I		Period II		Period III	
	Amount per day gm.	Dry matter per day gm.	Amount per day gm.	Dry matter per day gm.	Amount per day gm.	Dry matter per day gm.
Fish meal	150	129·8	210	183·5	150	132·5
Middlings	500	435·0	700	616·3	500	438·3
Maize meal	700	602·4	980	853·9	700	604·8
Molasses-sugar beet pulp	500	446·0	—	—	—	—
Dried sugar-beet pulp ...	—	—	—	—	500	415·7

At the end of the second period, no difficulty of any kind was encountered in accustoming the pigs to the ration containing dried sugar-beet pulp. By this time, however, the animals weighed 222 and 223 lb. respectively and appeared better able to deal with such bulky food than had been the case at the beginning of the first period.

The experimental periods, during which the excreta were quantitatively collected for analysis, were of 10 days' duration. The harness and metabolism crates which were employed to render possible the separate collection of urine and faeces have been described in an earlier publication (3). The various foods were sampled for analysis at the beginning of the feeding trials. Prior to each digestion period, the rations for the whole period were weighed into paper bags and at the same time samples of the foods were taken for determinations of moisture content.

The composition of the different feeding stuffs is shown, on the basis of dry matter, in Table II. From the data recorded in Table I, it is possible to calculate the moisture contents of the feeding stuffs at the time of weighing out the rations.

Table II. *Composition of feeding stuffs (on basis of dry matter).*

	White fish meal %	Middlings %	Maize meal %	Molasses-sugar beet pulp %	Dried sugar-beet pulp %
Crude protein ...	68·72	17·46	10·52	9·67	10·16
Ether extract ...	3·10	5·37	5·52	1·10	0·60
N-free extractives ...	3·00	64·66	79·98	66·62*	64·00†
Crude fibre	—	7·85	2·29	17·29	21·39
Ash	25·18	4·66	1·69	5·32	3·85

* Including 16 per cent. sugar. † Including 1 per cent. sugar.

A summary of the digestion coefficients (mean for 2 pigs) which were obtained in the present trials is given in Table III. The results of a German trial with pigs on sugar-beet pulp carried out by Lehmann (4) in 1902 are also recorded, no other data in this connection having been discovered in the literature. For further comparison, the recent results of Woodman and Calton (1) for the digestibility of dried sugar-beet pulp when consumed by sheep are also included.

Table III. *Summary of digestion coefficients (mean for 2 pigs).*

	Present investigation with pigs				
	Basal ration (fish meal, middlings and maize meal) %	Molasses-sugar beet pulp %	Dried sugar-beet pulp %	Dried sugar-beet pulp (pigs) Lehmann (4) %	Dried sugar-beet pulp (sheep) Woodman and Calton (1) %
Organic matter ...	84·1	80·5	80·2	81	86·5
Crude protein ...	86·8	24·4	34·6	32	58·3
Ether extract ...	69·0	—	—	—	—
N-free extractives	88·2	89·2	87·2	91	91·1
Crude fibre ...	24·9	84·4	84·3	86	89·7

Comments on Table III.

The data in Table III lead to the conclusion that pigs are able to digest both dried sugar-beet pulp and molasses-sugar beet pulp almost as efficiently as sheep digest dried sugar-beet pulp, the digestion coefficient of the organic matter of sugar-beet pulp being 80·2 per cent. for pigs and 86·5 per cent. for sheep. Moreover, the same general characteristics are displayed by the results for both pig and sheep, namely, high digestion coefficients for the *N*-free extractives and crude fibre of the beet pulp and a low digestion coefficient for the protein constituent. For the probable explanation of the poor utilisation of the protein of sugar-beet pulp, an earlier publication of Woodman and Calton (1) should be consulted. As in the case of the sheep digestion trials, it was again found impossible in the present trials, owing to the low percentage of ether extract in sugar-beet pulp, to evaluate the digestibility of this

constituent. It will further be noted that the present results are in substantial agreement with those obtained in similar work by Lehmann in 1902(4).

The close agreement between the *mean* results for the molasses-sugar beet pulp and the dried sugar-beet pulp should not be unduly stressed, since the results for the individual pigs in the dried sugar-beet pulp period (see Appendix) displayed greater lack of harmony than is usual in this type of work, such discrepancies probably being due to the circumstances that the sugar-beet pulp formed only a small part, about 27 per cent., of the total experimental ration and that the digestion coefficients were determined by an indirect method of experiment. It will be seen, however, that the discrepancies do not affect the general conclusions which are to be drawn from the results of the trials. In the other periods, the results for the individual pigs displayed very satisfactory agreement.

The results of the present digestion trials are not in accordance with anticipation, in that they point to the ability of pigs to assimilate the fibre and carbohydrate of sugar-beet pulp to a degree almost equal to that displayed by sheep. As fibre digestion is brought about by the activity of bacteria, it is not difficult to understand why *ruminant* animals are able to deal very efficiently with the non-lignified fibre of sugar-beet pulp. That bacterial digestion of fibre is also possible in swine, however, is clear from the results of numerous digestion trials. In the basal period of the present investigation, for instance, almost 25 per cent. of the fibre in the fish meal-middlings-maize meal mixture was assimilated. In similar trials carried out in this Institute, it has been demonstrated that pigs are able to digest the fibre in barley meal(3), maize meal(5) and middlings(6) to the extent of 11, 23 and 35 per cent. respectively.

The capacity of pigs to deal with fibre is usually regarded as very limited. The present results, however, show that this capacity is dependent on the nature of the fibre in the feeding stuff, and that when the fibre is non-lignified (that is, when it is composed mainly of cellulose) then such fibre may be digested to a very high degree, as is evidenced by the present fibre digestion coefficients, namely, 84·4 and 84·3 per cent. for molasses-sugar beet pulp and dried sugar-beet pulp respectively. Similar high digestion coefficients from pig-feeding trials are recorded by Kellner(7) for the fibre of mangolds (79 per cent.) and of dried potato slices (80 per cent.). In a digestion trial carried out by the writers with pigs on whole sugar beet, the fibre was found to be digested to the extent of 90 per cent(8).

Besides being able to deal efficiently with the non-lignified fibre of roots and their by-products, it is evident that pigs can also assimilate to a high degree certain types of carbohydrate which require the intervention of bacteria for their breakdown and digestion. The N-free extractives of sugar-beet pulp are composed mainly of pectose. As no enzyme has been detected in the secretions of the digestive tracts of animals which has the power of breaking down the pectose complex, it is assumed that the pectose in beet pulp is rendered available by the digestive action of bacteria (1). On these grounds, it was predicted that the N-free extractives, which comprise about two-thirds of the total dry matter of sugar-beet pulp, would only be poorly utilised by pigs. The contrary was found actually to be the case. The results recorded in Table III show that the N-free extractives of sugar-beet pulp are digested by pigs almost as efficiently as by sheep. It is noteworthy that the N-free extractives, consisting mainly of starch, in the basal ration were no better digested by the pigs than were the N-free extractives of the molasses-sugar beet pulp and the dried sugar-beet pulp.

During the carrying out of the digestion trials on the two kinds of beet pulp, daily tests were made for reducing sugars in the urine of the pigs. The tests were invariably negative, and it may therefore be concluded that no pentose sugar was being lost in this manner when the pigs were subsisting on rations containing dried sugar-beet pulp and molasses-sugar beet pulp, feeding stuffs which give rise, among other compounds, to arabinose during digestion. This observation is in agreement with that made in the earlier investigation with sheep.

II. Farm trials.

It has been shown in the foregoing section that pigs are able to digest sugar-beet pulp, whether in the form of the simple dried product or in admixture with beet molasses, with a high degree of efficiency. From the simple standpoint of digestibility, therefore, it would appear justifiable to conclude that sugar-beet pulp should constitute a satisfactory food for inclusion in the pig's dietary. Other points remain to be considered, however, before such a conclusion can be accepted with safety. Since digestion of beet pulp appears to be mainly bacterial in character, it must be shown that the products of such bacterial digestion in the pig are capable of productive utilisation in the body of the animal. It is also possible that the bulkiness of sugar-beet pulp, although not a prohibiting factor in the feeding of ruminant animals, may seriously limit the usefulness of this feeding stuff in the nutrition of swine. In

order to throw light on these further questions, a large-scale feeding trial was carried out at the Animal Nutrition Piggeries on the University Howe Hill Farm, in which the progress of pigs on a normal diet was compared with that of other pigs on a diet in which part of the barley meal had been replaced by dried sugar-beet pulp.

The general plan of the feeding trial is indicated in Table IV.

Table IV. *General plan of farm trial.*

Group of animals ...	I	II	IV
Feeding period I ...	Dried sugar-beet pulp	Control	Control
Feeding period II ...	Control	Control	Control
Feeding period III ...	Control	Dried sugar-beet pulp	Control

Selection of animals. Pre-experimental period.

On the 24th September, 1928, 66 pigs were received at the University Farm. Only 42 of these were retained for experimental purposes, however, the remainder being rejected as unsuitable on various grounds. The animals had been running out on grass practically all their lives. Their skin condition was poor and difficulty was experienced in getting them to feed adequately. They were also continually scratching and rubbing. From September 24 to October 24, the pigs were fed with as much as they could consume of a mixture consisting of 35 parts barley meal, 15 parts flaked maize, 15 parts maize meal, 25 parts middlings, 5 parts each of extracted soya bean meal and fish meal and 1 part of a suitable mineral mixture. They received no green stuff or other succulent food until the commencement of the experiment. During the week before the actual start of the comparative trials, all the animals received Mixture 45 (see Table V).

On October 8, the pigs were "wormed" with a mixture of 5 per cent. Oil of Chenopodium and 95 per cent. castor oil by volume, and on October 11 they received a first inoculation of Swine Erysipelas Vaccine, followed by a second treatment a week later[1]. On October 15, the animals were sorted into four experimental groups, the following factors being taken into consideration: Sex, age, weight, breeding and weight gains in the 17 days since September 24. There were 9 pigs in each group at this date, but it became necessary to withdraw certain of the animals before or immediately after the beginning of the experiment on account of bad condition and slow growth. On November 1, the initial day of the experiment, the selected pigs, although in fairly good condition, were not really thriving.

[1] The authors wish to acknowledge their indebtedness to the Institute of Animal Pathology, Cambridge, for the carrying out of these operations.

Housing and weighing.

The groups of pigs were housed in a well-lighted piggery with concrete-floored pens and dunging passages. Wheat straw was used as litter. The comfort of the animals was increased by laying large thatched hurdles half-way across the top of each pen.

The pigs were weighed once a week before the morning feed. At the beginning and end of each feeding period, however, weighings were made on three successive mornings and averaged.

Rations and Feeding.

The experiment was started on November 1, 1928, and ran for 16 weeks, ending on February 21, 1929. It was divided into three periods: Period I up to the end of the seventh week, during which Group I was on the sugar-beet pulp ration and Groups II and IV were on the control rations; Period II from the seventh to the eleventh week, when all the groups were receiving the control rations; Period III from the eleventh to the sixteenth week, when Group II was placed on the sugar-beet pulp ration, and Groups I and IV were still receiving the control rations. The pigs in Group III were used over this time for testing the value of whole sugar beet in the rations of swine (8).

Table V. *Summary of rations.*

Control rations.

	Mixture 45 (up to 150 lb. live weight)	Mixture 46 (150 lb. live weight to slaughter)
Barley meal ...	65 parts	75 parts
Middlings ...	25 ,,	20 ,,
Fish meal ...	10 ,,	5 ,,

Sugar-beet pulp rations.

	Mixture 47 (up to 150 lb. live weight)	Mixture 48 (150 lb. live weight to slaughter)
Dried sugar-beet pulp	19 parts	19 parts
Barley meal	48 ,,	58 ,,
Middlings	25 ,,	20 ,,
Fish meal	10 ,,	5 ,,

Dried sugar-beet pulp was fed to an amount equivalent to one-sixth of the meal ration, the replacement of barley meal being effected on the basis of 1 lb. of dried sugar-beet pulp being equal to 0·9 lb. of barley meal (1). The meals were mixed up weekly, and the day's rations were weighed out at 10 o'clock in the morning and allowed to soak in 3 times the weight of water until 4.15 p.m. At this time, half the ration was fed to the pigs, the remainder being fed at 7.15 on the following morning. The rations were adjusted to the appetites of the animals in order to

avoid complications arising from food residues. Half a pound of succulent food per pig per day was given at midday. From November 1 to December 26, this consisted of kohlrabi, then of swedes up to February 13 and finally of mangolds until February 20. The mean percentages of dry matter in the barley meal, dried sugar-beet pulp, fish meal and middlings were 84·2, 77·3, 84·6 and 87·5 per cent. respectively.

Condition of pigs throughout feeding trial.

Group I. Difficulty was experienced during the first week in getting this group on to the experimental diet, which then contained dried sugar-beet pulp. Some of the pigs showed a tendency to scour. In the subsequent weeks the animals consumed the food with greater zest, though their skin and hair became progressively dirtier and duller. At the end of the seventh week the condition and appearance were so unsatisfactory that it was deemed advisable to omit the dried sugar-beet pulp from the ration. Within a week of the pigs being placed on the control ration, their improvement was obvious. They began to gain live-weight rapidly, were keener on their food and were generally brighter and more active. At the conclusion of the trial the pigs were in fairly good condition, though their skins had not the best possible appearance.

During the final 10 days of the experiment, the mixture of meal and water was frequently frozen in the mornings. This, of course, applied to the feeding of all the groups. It was observed that one pig (No. 24) did not lose condition at all when on the sugar-beet pulp ration and was thriving throughout the whole period of the experiment.

Group II. During the first few weeks of the experiment, the pigs in this group, which were receiving the control ration, were in better condition than the animals in Group I, although their skin and hair were not so bright as might be desired. Their skin appearance improved quickly, however, and the pigs maintained a normal appearance until about the twelfth week, when they were put on a ration containing dried sugar-beet pulp. From this point their appetite diminished abruptly, and in 4 days the total food consumed by this group per day fell from 44 to 21 lb. A marked loss of condition was apparent at the end of the second week on the sugar-beet pulp diet. The pigs were restless, their coats were dull and their skin was greasy. At no stage, however, was the appearance of their skin so bad as was the case with the Group I animals, when the latter were receiving the sugar-beet pulp diet. It was noted that the animals consumed their small midday allowance of succulent food with greater zest while they were on the sugar-beet pulp diet. It was also

observed that, despite the loss of external condition, the dung of the animals was as firm as that from the animals on the control ration.

Group IV. This group of pigs was kept on the control diet throughout the whole experiment. In the earlier stages of the experiment, the skin and hair condition of this group was no better than with Groups I and II. The animals went back slightly in condition, but regained this from the ninth week onwards. All through the experiment they were decidedly restless and towards the end became rather "fussy" with their food. On the final date, they were noted as a good even group of animals with good coats, but somewhat unfinished from the bacon point of view.

Results of farm trials.

The results obtained with the three groups of pigs in Periods I, II and III are given in Table VI.

Table VI. *Summary of live-weight gains and food consumption.*

Period I (November 1, 1928 to December 19, 1928).

Group of animals	I	II	IV
Ration	Dried sugar-beet pulp ration Mixture (47)	Control (45)	Control (45)
No. of pigs averaged	8	7	8
Average initial weight in lb.	69	69·1	64·6
Average final weight in lb.	105·4	122·9	111·2
Average gain during period in lb. ...	36·4	53·8	46·6
Average daily gain in lb.	0·74	1·10	0·95
Average meal consumption per day in lb.	3·03	4·16	3·87
Average meal consumption per lb. of live-weight increase	4·09	3·79	4·07

Period II (December 20, 1928 to January 16, 1929).

Ration	Control (45)	(Control (45)	Control (45)
No. of pigs averaged	8	7	8
Average initial weight in lb.	105·4	122·9	111·2
Average final weight in lb.	148·5	161·8	150·0
Average gain during period in lb. ...	43·1	38·9	38·8
Average daily gain in lb.	1·54	1·39	1·39
Average meal consumption per day in lb.	5·73	5·46	5·20
Average meal consumption per lb. of live-weight increase	3·72	3·93	3·74

Period III (January 17, 1929 to February 20, 1929).

Ration	Control (46)	Dried sugar-beet pulp (48)	Control (46)
No. of pigs averaged	8	7	8
Average initial weight in lb.	148·5	161·8	150·0
Average final weight in lb.	204·6	194·3	204·1
Average gain during period in lb. ...	56·1	32·5	54·1
Average daily gain in lb.	1·60	0·93	1·55
Average meal consumption per day in lb.	7·55	5·29	6·87
Average meal consumption per lb. of live-weight increase	4·72	5·69	4·43

It will be noted that during the first period the pigs in Group I, which were then on the sugar-beet pulp diet, made smaller live-weight gains than the animals in either of the Control Groups II and IV. This difference, however, was not due to a deficiency of food value in the sugar-beet pulp, but to the circumstance that the inclusion of this feeding stuff in the ration led to a smaller consumption of food by the Group I pigs. This is borne out by the fact that the amount of meal required per lb. of live-weight increase among the pigs in Group I was the same as for the pigs in Group IV and not significantly greater for the pigs in Group II.

During the second period, the pigs in Group I, which had then been brought on to the control diet, displayed a distinct recovery, and their rate of live-weight increase was 10 per cent. better than that for the animals in Groups II and IV, which were also on the control diet. There was no significant difference between the groups in this period in respect of economy of food conversion, *i.e.* meal required per lb. of live-weight gain.

During Period III, the Group II pigs, which were now subsisting on a diet containing dried sugar-beet pulp, showed only 60 per cent. of the gains exhibited by the animals on the control diet in Groups I and IV. The introduction of sugar-beet pulp again led to a pronounced falling-off of the amount of food consumed, and this circumstance was mainly responsible for the slowing-up of the rate of growth in the pigs in Group II. In contrast with the observations in Period I, however, the economy of food conversion in Group II was distinctly lower than that for the groups on the control diet, the amounts of meal required per lb. of live-weight increase being 4·72, 5·69 and 4·43 lb. for Groups I, II and IV respectively. It should be borne in mind, however, that in the fortnight following the change-over to the sugar-beet pulp diet, the pigs in Group II suffered a severe setback owing to pronounced loss of appetite. It is probable that if the experiment had been extended some weeks beyond the end of this short 5 weeks' period, the differences between the economies of food conversion for the sugar-beet pulp and the control groups would not have been so pronounced.

Quality and carcase results.

Groups I and II were weighed alive at 7.30 a.m. on February 26th, 1929, killed on the 27th and the carcases were weighed and graded on the morning of the 28th. Group IV was weighed alive on the morning of the 23rd, killed on the 25th and graded on the 26th. The carcases were treated in exactly the same way as the pigs received at the slaughter

centres of the East Anglian Pig Recording Scheme (9), with the sole difference that, as the pigs were sold as pork, it was not possible to obtain curing weight. The grading was largely based on thinness of back fat, but length, general suitability and finish were also taken into consideration.

On this basis, Group IV (control ration) provided the best quality pigs, followed by Group I (dried sugar-beet pulp ration in Period I) and lastly Group II (dried sugar-beet pulp ration in Period III).

CONCLUSIONS.

Pigs are able to digest dried sugar-beet pulp and molasses-sugar beet pulp to an extent very little inferior to that to which ruminant animals are able to digest these feeding stuffs. Despite this finding, however, it is clear that sugar-beet pulp, although a good source of carbohydrate for sheep, bullocks and dairy cows, is by no means an entirely suitable food for pigs. Its inclusion in pig rations, even to the moderate extent of the equivalent of one-sixth of the ration, causes the mixed food to be very bulky after the usual soaking in water. This leads to difficulties in securing satisfactory consumption of the food, and it is found that pigs, in consequence of the well-known difficulty they experience in dealing with bulky foods, are unable to consume as big a ration as is possible when sugar-beet pulp is omitted.

For this reason, the inclusion of sugar-beet pulp in the rations of pigs depresses the rate of live-weight increase, although the effect on the economy of food conversion is of much smaller account. Although sugar-beet pulp is highly digested by pigs, its bulk prevents its being a suitable food for use in more than small quantities when it is desired to make pigs into bacon as quickly as possible. There seems no reason, however, why it should not be used in moderate quantities as a food for breeding stock, or for pigs which are not being fed to their maximum appetite with a view to obtaining early maturity.

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Appendix.

Digestion tables.

Pig VI

Period I	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	*N*-free extractives gm.	Crude fibre gm.	Ash gm.
Fish meal	129·80	97·12	89·20	4·02	3·90	—	32·68
Middlings	435·00	414·73	75·95	23·36	281·27	34·15	20·27
Maize meal	602·40	592·22	63·37	33·25	481·80	13·80	10·18
Molasses-sugar beet pulp	446·90	423·13	43·22	4·92	297·72	77·27	23·77
Total consumed	1614·10	1527·20	271·74	65·55	1064·69	125·22	86·90
Total voided	306·79	263·78	64·62	25·32	125·36	48·48	43·01
Total digested	1307·31	1263·42	207·12	40·23	939·33	76·74	43·89
Digested from basal food	946·48	921·26	195·82	41·24	672·63	11·57	25·22
Digested from molasses pulp	360·83	342·16	11·30	—	266·70	65·17	18·67
Dig. coefficients of molasses pulp, %	80·74	80·86	26·15	—	89·58	84·34	78·54
Period II							
Fish meal	183·50	137·30	126·10	5·69	5·51	—	46·20
Middlings	616·30	587·58	107·60	33·10	398·50	48·38	28·72
Maize meal	853·90	839·47	89·83	47·14	682·95	19·55	14·43
Total consumed	1653·70	1564·35	323·53	85·93	1086·96	67·93	89·35
Total voided	312·66	259·01	46·31	27·48	133·68	51·54	53·65
Total digested	1341·04	1305·34	277·22	58·45	953·28	16·39	35·70
Dig. coefficients of basal food, %	81·09	83·44	85·69	68·02	87·70	24·13	39·95
Period III							
Fish meal	132·50	99·14	91·05	4·11	3·98	—	33·36
Middlings	438·30	417·88	76·53	23·54	283·40	34·41	20·42
Maize meal	604·80	594·58	63·62	33·39	483·72	13·85	10·22
Dried sugar-beet pulp	415·70	399·70	42·24	2·49	266·05	88·92	16·00
Total consumed	1591·30	1511·30	273·44	63·53	1037·15	137·18	80·00
Total voided	299·72	251·36	56·50	22·46	121·12	51·28	48·36
Total digested	1291·58	1259·94	216·94	41·07	916·03	85·90	31·64
Digested from basal food	953·29	927·72	198·12	41·52	676·43	11·65	25·57
Digested from sugar-beet pulp	338·29	332·22	18·82	—	239·60	74·25	6·07
Dig. coefficients of sugar-beet pulp, %	81·38	83·12	44·55	—	90·06	83·50	37·94

Pig VII

Period I	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	*N*-free extractives gm.	Crude fibre gm.	Ash gm.
Total consumed	1614·10	1527·20	271·74	65·55	1064·69	125·22	86·90
Total voided	301·59	253·03	61·25	23·81	120·35	47·62	48·56
Total digested	1312·51	1274·17	210·49	41·74	944·34	77·60	38·34
Digested from basal food	959·56	935·52	200·71	42·46	680·02	12·33	24·04
Digested from molasses pulp	352·95	338·65	9·78	—	264·32	65·27	14·30
Dig. coefficients of molasses pulp, %	78·98	80·04	22·63	—	88·78	84·47	60·16
Period II							
Total consumed	1653·70	1564·35	323·53	85·93	1086·96	67·93	89·35
Total voided	294·20	238·87	39·38	25·75	123·28	50·46	55·33
Total digested	1359·50	1325·48	284·15	60·18	963·68	17·47	34·02
Dig. coefficients of basal food, %	82·21	84·73	87·83	70·03	88·66	25·72	38·08
Period III							
Total consumed	1591·30	1511·30	273·44	63·53	1037·15	137·18	80·00
Total voided	314·32	260·40	60·00	22·36	128·97	49·07	53·92
Total digested	1276·98	1250·90	213·44	41·17	908·18	88·11	26·08
Digested from basal food	966·46	942·09	203·06	42·75	683·87	12·41	24·37
Digested from sugar-beet pulp	310·52	308·81	10·38	—	224·31	75·70	1·71
Dig. coefficients of sugar-beet pulp, %	74·70	77·26	24·58	—	84·31	85·13	10·69

Note. The care of the animals during the digestion trials was in the hands of Messrs V. Thurlbourn and C. Bendall. In the farm trials, Mr E. A. Porter had charge of the pigs.

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THE VALUE OF WHOLE SUGAR BEET IN THE NUTRITION OF SWINE.

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Introduction.

Though it is neither customary nor perhaps desirable to utilise sugar beet for feeding purposes, it is conceivable that circumstances might arise occasionally when a farmer would be desirous of feeding the whole or part of his beet crop. This is indicated by the fact that enquiries as to the value of sugar beet for pigs are received from time to time. During the carrying out of the investigation into the value for pigs of dried sugar-beet pulp and molasses-sugar beet pulp, an account of which work is given in this issue of the *Journal*(1), the opportunity was taken of making a similar study of whole sugar beet. A dual investigation was made, consisting of a digestion trial under the conditions of the metabolism room and a large-scale feeding trial under ordinary farm conditions. It was hoped that the evidence from this twofold line of enquiry would not only settle the question of the value of sugar beet in the feeding of pigs, but also throw light on the subject of the value of root crops in general for swine.

I. Digestion trial.

The same two Large White hogs were employed for this purpose as were used in the sugar-beet pulp trials, the whole sugar beet period preceding the period in which molasses-sugar beet pulp was fed. At this stage the animals weighed 160 and 165 lb. respectively. The analytical period, which followed the usual preliminary period of feeding, was of 10 days' duration. The details of the experimental ration are shown in Table I. For the particulars concerning the determination of the digestibility of the basal food, the previous publication(1) should be consulted.

In the preliminary stages of the trial, the washed sugar beets were grated by a special machine for feeding to the pigs. It was found, how-

ever, that the grated roots always contained some lumpy material which was not consumed by the animals. In order to ensure complete consumption of the ration, therefore, it was found necessary to put the grated sugar beet through a mincing machine, care being taken to lose none of the juice. The mixture of fish meal, middlings, maize meal and minced sugar beet was allowed to soak overnight in 5000 c.c. of water and was fed to the pigs in four meals per day. The ration was consumed eagerly and cleanly. The faeces of the pigs were somewhat soft when the animals first went on to the experimental diet, but had attained a satisfactory consistency by the beginning of the analytical period, no difficulty being experienced in securing quantitative collection of the excreta.

Table I. *Details of experimental ration.*

	Amount per day gm.	Dry matter per day gm.
Fish meal	150	128·7
Middlings	500	434·2
Maize meal	700	596·5
Sugar beet	2800	732·2

Daily determinations were made of the dry matter content of the minced sugar beet as weighed out for the pigs. The extreme values were 25·60 and 26·92 per cent., with a mean percentage of dry matter for the whole period of 26·15 per cent. The residues from the dry matter determinations were saved for making a composite sample for complete analysis.

Table II. *Mean composition of whole sugar beet (on basis of dry matter)*.*

	%
Crude protein	5·99
Ether extract	0·79
N-free extractives ...	84·73
Crude fibre	4·44
Ash	4·05

* For composition of other feeding stuffs employed in the trial, see previous paper (1).

During the course of the digestion trial, the sugar content, determined on 10 average roots by the usual factory method, was found to be 16·8 per cent. Daily tests of the urine of the pigs showed that no reducing sugar was being excreted by this path. Over a period of 14 days on the experimental ration, Pig VI gained 17 lb. and Pig VII 15 lb. in weight. The mean daily nitrogen balances during the analytical period were + 14·81 gm. and + 14·52 gm. respectively.

Table III. *Digestibility of whole sugar beet**.

Pig VI

Consumed:	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
Fish meal	128·70	96·29	88·44	3·99	3·86	—	32·41
Middlings	434·20	413·97	75·81	23·32	280·75	34·09	20·23
Maize meal	596·50	586·42	62·75	32·93	477·08	13·66	10·08
Sugar beet	732·20	702·54	43·86	5·78	620·39	32·51	29·66
Total consumed	1891·60	1799·22	270·86	66·02	1382·08	80·26	92·38
Total voided	269·77	227·34	55·94†	26·19	106·00	39·21	42·43
Total digested	1621·83	1571·88	214·92	39·83	1276·08	41·05	49·95
Digested from basal food	940·16	915·10	194·52	40·98	668·08	11·52	25·06
Digested from sugar beet	681·67	656·78	20·40	—	608·00	29·53	24·89
Dig. coefficients of sugar beet, % ...	93·10	93·48	46·51	—	98·00	90·83	83·92

Pig VII

Total consumed (as above)	1891·60	1799·22	270·86	66·02	1382·08	80·26	92·38
Total voided	278·26	234·78	56·81†	29·50	109·52	38·95	43·48
Total digested	1613·34	1564·44	214·05	36·52	1272·56	41·31	48·90
Digested from basal food	953·14	929·26	199·37	42·19	675·42	12·28	23·88
Digested from sugar beet	660·20	635·18	14·68	—	597·14	29·03	25·02
Dig. coefficients of sugar beet, % ...	90·17	90·41	33·47	—	96·25	89·30	84·35
Mean digestion coefficients, % ...	91·6	91·9	40·0	—	97·1	90·1	84·1

* For the details of the basal period, see previous paper(1).

† The daily output of crude protein is calculated on the basis of the nitrogen content of the *fresh* faeces.

Comments on Table III.

It will be seen from Table III that swine are able to digest the nutrient matter in sugar beet with a high degree of thoroughness. This fact is brought out more clearly by the comparative data recorded in Table IV.

Table IV. *Summary of digestion coefficients obtained in digestion trials with swine.*

	Sugar beet (present trial) %	Dried sugar-beet pulp(1) %	Maize meal(2) %	Flaked maize(2) %	Barley meal(3) %	Dried potato slices(4) (Kellner) %	Mangolds (4) (Kellner) %
Organic matter	91·9	80·2	87·8	95·4	81·7	90·0	90·0
Crude protein	40·0	34·6	80·1	95·5	81·7	54·0	55·0
Ether extract ...	—	—	60·5	44·8	70·0	—	—
N-free extractives	97·1	87·2	92·0	97·1	88·7	94·0	98·0
Crude fibre ...	90·1	84·3	35·3	30·5	10·8	80·0	79·0

Table IV reveals the fact that the organic matter in roots and tubers in general is digested by swine very efficiently, the value obtained for sugar beet in the present trial, namely, 91·9 per cent., being very similar to the corresponding values for mangolds and dried potato flakes obtained in old German trials. Further points of similarity between

the utilisation by pigs of sugar beet, potatoes and mangolds are discernible in the high digestion coefficients for the N-free extractives and the fibre and the relatively low values for the crude protein. Moreover, in none of these cases has it been found possible to determine the degree to which the ether extract is digested.

It should also be noted that in respect of the digestibility of organic matter and N-free extractives, sugar beet compares well with such a highly digestible food as flaked maize. Whereas, however, the crude protein of sugar beet is only poorly digested, that of flaked maize is assimilated almost completely. On the other hand, the fibre of sugar beet is very much more digestible than that of flaked maize.

The amounts of digestible nutrients in 100 lb. of the dry matter of sugar beet, barley meal and maize meal are shown in Table V.

Table V. *Digestible nutrients in sugar beet, barley meal and maize meal.*

100 lb. dry matter of food contains:	Sugar beet (present trial) lb.		Barley meal(3) lb.		Maize meal(2) lb.	
Digestible crude protein	2·40		10·04		8·77	
Digestible ether extract	—		0·61		3·10	
Digestible N-free extractives ...	82·27	86·27	69·27	69·87	73·42	74·22
Digestible crude fibre	4·00		0·60		0·80	
Digestible organic matter	88·67		80·52		86·09	

The dry matter of sugar beet is shown in Table V to be almost identical with the dry matter of maize in respect of its content of total digestible organic matter, and somewhat superior in this regard to the dry matter of barley. Considered as a source of digestible carbohydrate, for which purpose these feeding stuffs would be employed in pig-feeding, sugar beet is superior, on the basis of dry matter, to both maize and barley.

It is clear from the results that it will be safe to assume in practice that 1 lb. of the dry matter of barley can be replaced by 1 lb. of the dry matter in sugar beet. Assuming the values 25 and 85 for the average percentages of dry matter in sugar beet and barley respectively, it follows that 1 lb. of barley can be replaced by 3·4 (say $3\frac{1}{2}$ lb.) of sugar beet.

II. Farm trial.

The pigs used in this feeding trial were selected from the animals which had been secured for the purpose of the sugar-beet pulp trials reported in the preceding paper in this issue (1). Of the four groups into which these pigs were divided, Group III was fed on rations containing

grated sugar beet throughout the whole experiment, the progress of this group being compared with that of the control group, namely Group IV. The procedure in connection with the selection of the animals in Group III, as well as in respect of their treatment in the pre-experimental period and their weighing, housing and feeding, was precisely the same as has been described in the foregoing communication.

Rations and feeding.

The barley meal, middlings and fish meal used in making up the rations for Group III were drawn from the same consignments as were employed in the sugar-beet pulp trials. The average percentages of dry matter in these ingredients of the rations were 84·2, 87·5 and 84·6 per cent. respectively. The percentage of dry matter in the sugar beet, which had been grown on the University Farm, was determined week by week throughout the trial. The values varied from 24·15 to 26·91 per cent., with a mean value of 25·53 per cent. Determinations of sugar content, by the usual factory method, were made on November 30, 1928, and January 8, 1929, when the values 16·8 and 18·2 per cent. were obtained respectively.

Table VI. *Summary of rations.*

	Control rations.	
	Mixture 45 (up to 150 lb. live weight)	Mixture 46 (150 lb. live weight to slaughter)
Barley meal	65 parts	75 parts
Middlings	25 „	20 „
Fish meal	10 „	5 „
	Sugar beet rations.	
	Stage I (up to 150 lb. live weight)	Stage II (150 lb. live weight to slaughter)
Sugar beet (100 parts) Blood meal (1 part)	112½ parts	112½ parts
Barley meal	40 „	50 „
Middlings	25 „	20 „
Fish meal	10 „	5 „

It will be noted that the rations of Group III were computed by replacing 25 parts of barley meal (*i.e.* one-quarter of the total control ration) by an equivalent amount of a mixture composed of 100 parts of grated sugar beet and 1 part of blood meal. Assuming that sugar beet contains 0·8 per cent. of digestible crude protein and 15 per cent. of starch equivalent (5), then 112½ parts of such a mixture of sugar beet and blood meal contain approximately the same amount of starch equivalent and digestible protein as 25 lb. of barley meal.

In the preparation of the pig rations, the sugar beets were first cleaned, washed and drained. The weight of roots required was then put through an Andersen and Egevist root-grating machine. The blood meal and the required amount of the barley meal-middlings-fish meal mixture were next added, and the whole was well mixed, put through the machine again and kept in a wooden tub for use on the following day. Before feeding the mixture, an equal weight of water was stirred in.

The Andersen and Egevist root grater.

This machine consists of a hard wooden drum over which is stretched a galvanised plate, punctured by hardened steel points fitting into hard wooden blocks. The drum rotates against a fixed galvanised plate, the roots being forced between the drum and the fixed plate. In the case of sugar beet, the grated product has the appearance of soap flakes. Usually, however, a few lumps escaped being reduced to flakes. When large quantities of roots were being grated, however, the proportion of such lumpy material would be unimportant. In addition to sugar beets, the following roots were found to be grated satisfactorily by the Andersen and Egevist machine: red mangolds, swedes and kohlrabi.

Course of farm trial.

The progress of the control pigs in Group IV has already been described in the preceding paper(1). The animals in Group III (sugar beet diet) were somewhat "scoured" during the first fortnight of the experiment and food consumption tended to fall off. During the second week, however, each pig was given a dose of 2 oz. ground chalk. The faeces quickly improved to a normal consistency and remained so until the end of the trial. For the rest of the feeding period the animals were bright and active and, with the exception of a slight setback during the ninth week, displayed a keen appetite. At the conclusion of the experiment in the sixteenth week, Group III was the best group in the piggery in respect of skin and hair condition. The pigs in this group were also fatter and heavier in the shoulders than the animals in the control group. In the grading of the carcases after slaughter, it was concluded that as regards quality for the production of Wiltshire bacon, the grated sugar beet pigs were equal, or even slightly superior, to those fed on the normal control rations.

Results of farm trial.

The results of the trial are given in Tables VII and VIII.

Table VII. *Summary of live-weight gains* (16 *weeks' feeding period*).

Group of animals ...	III	IV
Ration	Grated sugar beet mixture	Control mixture
No. of pigs averaged	8	8
Average initial weight in lb. ...	68·9	64·6
Average final weight in lb. ...	220·8	204·1
Average gain during period in lb.	151·9	139·5
Average daily gain in lb.	1·36	1·25

Table VIII. *Food consumption and economy of food conversion.*

	Average food consumption per pig during whole 16 weeks' trial		Average food consumption per pig per 100 lb. of live-weight increase			
	Group III	Group IV	Group III		Group IV	
	lb. food	lb. food	lb. food	lb. dry matter	lb. food	lb. dry matter
Grated sugar beet (100 parts) plus blood meal (1 part)	638·7	—	420·5	107·35	—	—
Barley meal	251·4	397·8	165·5	139·34	285·2	240·14
Middlings	126·9	131·7	83·5	73·06	94·4	82·60
Fish meal	42·7	45·5	28·1	23·77	32·6	27·58
Total	1059·7	575·0	697·6	343·52	412·2	350·32

Dry matter consumption per 1 lb. live-weight increase Group III, 3·44 lb.
,, ,, ,, Group IV, 3·50 lb.

It will be noted from Tables VII and VIII that the grated sugar beet group gained 1·36 lb. per day as against 1·25 lb. for the pigs in the control group. For 1 lb. of live-weight increase, 3·44 lb. of dry matter was consumed by the animals on the sugar beet diet, compared with a consumption of 3·50 lb. of dry matter by the control pigs. As this difference is without significance, it may be concluded that the dry matter of the sugar beet ration was equal, weight for weight, to the dry matter of the control ration for the purpose of securing live-weight increase in swine. On an average, the pigs in Group III consumed, in addition to their meal allowance, 420·5 lb. of the mixture of grated sugar beet and dried blood (100 : 1) per 100 lb. of live-weight increase, whereas the control pigs, which received none of the sugar beet mixture, consumed, on an average, 119·7 lb. more barley meal, 10·9 lb. more middlings and 4·5 lb. more fish meal per 100 lb. of live-weight gain than was eaten by the Group III pigs. If the 4·2 lb. of dried blood in the 420·5 lb. of sugar beet mixture be cancelled against the extra middlings

and fish meal eaten by the control pigs (a cancellation which certainly favours the barley meal rather than the grated sugar beet) then it is fair to conclude that 416 lb. of sugar beet is able to replace 120 lb. of barley meal in the rations of swine. In other words, the pig-feeder who wishes to replace part of the barley meal in the rations of his pigs by sugar beet will be on the safe side by effecting the substitution on the following basis: 1 part barley meal $\equiv 3\frac{1}{2}$ parts by weight of grated sugar beet. It will be noted that this conclusion is in complete harmony with that drawn from the results of the digestion trials described in the first section of this paper.

Conclusion.

Whole sugar beet, suitably grated, may be used to replace barley meal up to 25 per cent. of the total ration in the production of bacon pigs. The substitution should be effected at the rate of $3\frac{1}{2}$ lb. of sugar beet to 1 lb. of barley meal.

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THE COMPOSITE CHARACTER OF THE SOIL PROFILE, ITS RELATION TO SOIL CLASSIFICATION.

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THE scheme of soil classification which finds most favour among scientists to-day has come from Russia and is the work of a band of brilliant and enthusiastic investigators whose labours have brought some order to what was otherwise chaos. The object of this communication is to endeavour to show how it is possible to modify the views of this school so as to make them apply to British conditions, and how the majority of British soils may be brought within the envisaged scheme.

This scheme depends fundamentally upon the view that the nature of the decomposition of natural silicates, of whatever character, and the type of organic matter decay that occurs are determined by the climatic forces which obtain during the time that the soil is being elaborated from the parent rock. Upon this basis it is clearly possible to imagine that any given set of climatic conditions will elaborate a definite soil form, known as the agrogeological profile, typical of that environment. This profile will show certain horizon differentiation into zones of leaching and zones of accumulation, and it is this development of horizon differentiation that constitutes the interest and importance of the natural section.

The view has been held that two extreme cases are possible, in one of which the forces concerned have not had sufficient time to bring about changes in the various horizons, and in the other of which the forces have been acting for so long that no further change is possible, or that, at any rate, the form characteristic of that set of conditions has been produced. These two types have been designated immature and mature respectively, and the difference appears to depend upon the time factor alone. It has been the custom to consider all soils which occur in the same climatic region and which manifest this sharp horizon differentiation as mature soils, and those which do not show this characteristic structure as immature, and to imagine the latter as passing gradually from this stage to a stage of maturity in which the full soil profile can be readily distinguished.

It would appear that the starting-point of the Russian work, in so

far as the cooler and moister parts of that country were concerned, was a type which is very widely distributed and has come to be known as *podsol*. The other types appear to have been distinguished largely by contrast with this, so that this soil with its sharply defined horizons becomes, as it were, the standard with which other profiles have been contrasted and compared. Although this soil is widely distributed and occurs in large areas in the continent of Europe, its economic importance is not large owing to its infertility under agricultural, and even under sylvicultural, conditions.

Because of the importance attached to this type of soil as a standard of comparison it is worth while considering in some detail a characteristic profile of this kind, and enquiring what are the factors which have led to its development in a mature state.

In this type of soil the zones are well marked; the *A* zone, or the zone of leaching, contains some humus, usually well distributed in depth, and some organic constituents, bleached owing to the removal of iron, giving rise to the characteristic ash-like appearance of this horizon which has been responsible for its name. The *B* zone, or that of accumulation, may be characterised by a large quantity of humus, or of iron and aluminium oxides, or of both, and may form a layer impervious to air and water. The relative amount of humus and of iron and aluminium oxides in this zone appears to depend partly upon local conditions and partly upon age. It may generally be supposed that the older this layer is the more it is characterised by sesquioxides of iron and aluminium.

The conditions which have led to this horizon differentiation depend upon two things—a stream of percolating water, and some factor slowing down the rate of decomposition of the organic matter on the surface. It appears almost certain that the order of events is of this kind: (1) the first effect of a stream of percolating water, containing, as it does, small quantities of carbon dioxide, must be to remove continuously the exchangeable bases from the clay and humus portions of the soil; (2) this will continue until the content in exchangeable calcium is so low that the soil reaction becomes markedly acid, and (3) the concentration of the soil water in soluble substances becomes so low that bacterial action is slowed down and humus formation and decomposition is retarded. (4) At this point humus becomes dispersible and exerts a protecting effect upon iron and aluminium hydroxides so that (5) all three will tend to move throughout the upper regions of the soil.

The point that needs emphasis is that only when the soil is exhausted of its exchangeable bases and when the concentration of the soil water

is very low does this migration of humus and of associated sesquioxides occur. The formation of an accumulation, or B, zone may depend upon a variety of causes, partly physical and partly chemical, but is in a sense accidental, and may therefore be either well defined or hardly discernible, in which case it may be assumed that the migrating substances have been carried away in the underground water system. It thus appears that the one condition really necessary for a podsol structure to be established in the surface horizons is a stream of percolating water passing through an A_1 horizon in which organic matter decomposition is slowed down.

In order to consider the effect of a given rainfall in a particular area, it is obvious that not only the total rainfall must be taken into consideration, but also its distribution, as a high rainfall concentrated in a period of short duration would exert a greater leaching effect than the same rainfall extended over a longer period. That this is true is indicated by the conditions that lead to typical lateritic decomposition. The effect of this percolating water is also complicated by the fact that there is a considerable amount of evaporation from the surface of the bare soil, and, when the soil is carrying any kind of vegetation, absorption by, and transpiration through, the plant has to be taken into account so that the effect of 30 in. of rainfall may be quite different under different conditions. It is not only climate and vegetation, however, that will exert a controlling effect upon soil development, for such an important factor as the rate of water percolation will be determined by the texture of the soil particles and by the nature of the parent material. It would therefore appear that in addition to the ordinary effects of rainfall, temperature, etc., which can be summed up in the word climate, there must also be taken into account a *soil climate* depending upon such factors as the nature of the parent rock, the texture of its particles, and the extent to which it is rapidly permeable to water.

Up to the present time it has been the practice to regard the soil profile as a whole, but a further consideration will show that in reality it is properly regarded as consisting of a number of superposed profiles which are interdependent. Work carried out in the author's laboratory at Oxford by Mr Smedley, has shown quite clearly that there is found in soils, even under the climatic variations existing round Oxford, a horizon differentiation in soluble substances, that is in exchangeable bases, constituting a definite profile. The soils under examination showed what the author proposes to call a *solubility profile* varying according to the conditions under which the sample was taken. When the sample was

taken after a wet spell the content in exchangeable bases and the degree of saturation of the soil increased with depth, while after a spell of dry weather, when evaporation from the surface had been active, the highest concentration was found at or near the surface layer. Thus, in considering the production in any locality of a mature total profile, the influence of this solubility profile in modifying conditions has to be taken into account.

The striking effect of a period of dry weather will thus be to restore to the surface layers of the soil substances which had been removed by the previous percolation stream, and the effect will be intensified should the dry weather persist long enough, or the temperature be high enough, to cause partial dehydration of some of the soil colloids. Recent work carried out by Mr H. G. Coles in association with the author has shown that the increase in solubility of certain ions on dehydration of the soil is very considerable, so that this effect would be added to the merely mechanical effect should the dry weather persist. Dehydration also causes an increase in the acidity of the soil and, in most cases, a decrease in the exchangeable base content, so that on exposure to subsequent leaching the surface horizons have become poorer than before. This action appears to be only slowly reversible.

But it is not only in this direction that the complexity of the profile may be sought. It may be further considered to be built up of a *skeletal profile*, consisting of silica and silicates of iron and aluminium oxides, which represents the most permanent material and in a portion of which least change will take place, but the chemical and physical nature of which, in so far as its silica content is concerned, may be of controlling importance.

Added to this, and in some respects and under some conditions controlled by this, is what may be termed the *organic profile*, depending upon the nature and amount of humus substances formed by the vegetation.

If the typical podsol profile be now examined from this triple point of view its characteristics may be stated in the following terms:

Solubility profile. Hardly distinguishable, total content of exchangeable bases low but increasing with depth especially in the B horizon.

Skeletal profile. Iron and aluminium low in A horizon, but high in B horizon; silica and silicates showing possibly the same accumulation.

Organic profile. Normal vertical distribution in A_0, A_1, very low in the rest of A, showing accumulation in B horizon; normal diminution due to oxidation throughout B.

A normal distribution would mean in the case of the solubility and skeletal profiles a uniform distribution throughout the section, and in

the case of the organic profile a distribution decreasing with depth in the zone occupied by plant residues.

Bearing this profile complexity in mind it becomes possible to indicate soil type characters by the dominance of one profile over the others in the whole, thus, for example, in saline and alkaline soils the solubility profile is so dominant that the development of the other constituent profiles is determined entirely by this one. In podsol types the humus profile is the dominant one and, in the comparative absence of the solubility profile, controls the development of the skeletal profile which is characteristic of the total environment. In laterite it would appear that skeletal and solubility profiles between them build up the typical section.

It is now possible to explain the distribution of soils showing podsol characteristics in Great Britain. They occur commonly in the north and west, more sparingly in the south-west, and rarely in the south and east; they are thus found under conditions of rainfall varying in amount from 20 in. to 80 in. per annum.

It is suggested that this distribution is determined not only by the rainfall but by the extent to which the solubility profile, as defined above, controls the dispersion of other substances and consequently the nature of the skeletal and humus profiles. In the south and east of England, where the rainfall is low, the solubility profile is only negligible when the parent material consists almost entirely of quartz sand, and when this material is so deep or so coarse-grained that there is little or no return of dissolved substances to the surface. In all other cases, the solubility profile and the oscillation of its *B* horizon will prevent the dispersion necessary to secure the differentiation characteristic of the skeletal and organic profiles. In regions of higher rainfall a less extreme type of parent material is required to eliminate the solubility profile, and therefore the required conditions occur more frequently. In low rainfall districts, some of the sands of Norfolk and Suffolk and the Bagshot Sands of Hampshire and Surrey afford good examples. In regions of higher rainfall and lower summer temperature, and therefore less evaporation, podsolic conditions become increasingly common until they are found under almost all conditions of soil environment in the west of Ireland and Scotland.

The definite association of podsol building with both soil and atmospheric climate is due to the effect of the solubility profile upon the other constituent profiles of the soil which consequently remain normal. Except where the soil atmosphere and the physical and chemical character of

the parent material are such as to eliminate the effect of the solubility profile, the typical podsol structure is not produced. In those conditions, however, where the solubility *B* horizon, or zone of accumulation, moves down and up in the section according to season and rainfall then the skeletal and organic profiles remain normal and there is no tendency for an *A* horizon to be formed in either case. Soils of this type may then be said to represent material which would show podsol differentiation except for soil climatic environment, but which show a mature composite profile characteristic of soil and atmospheric climate.

It is of course possible to speak of these soils as immature, but only if some qualification be made indicative of their stability in their present environment. The author therefore proposes to introduce the term *stable immaturity* for all those soils which, under existing environment, do not show horizon differentiation considered typical of the region, but which, nevertheless, differ from immature soils in that they have reached a state of equilibrium controlled in the soils under discussion by the variation in the solubility profile.

The great areas of agricultural land in Great Britain and Ireland, varying much in their economic possibilities, form a group of brown soils which are, in the author's view, a striking example of this stable immaturity. In soils of this kind, oscillation of the *B* solubility horizon maintains normal skeletal and humus profiles, and thus ensures the uniform distribution of sesquioxides throughout the profile sometimes spoken of as characteristic of this type of soil. The nature of the parent material is such that there is initially only a slow loss of soluble substances, and much of what is lost in the winter is regained by the surface in the summer and in some cases there may even be an actual gain in soluble substances. In this environment also the parent material, and through it the skeletal profile, will continue to exert a maximum effect upon the character of the whole section. The author would thus consider the typical brown soils of these islands to be stable immature soils with their stability conditioned by oscillation of the solubility profile, the rate of movement of this depending upon the physical nature of the parent material.

If this view be accepted it will be desirable to examine afresh the profiles of brown soils from the composite point of view. There will probably be found to exist under certain conditions of atmospheric and soil climate a transition series, which in some years and under some conditions will show instability and a tendency for the horizon differentiation to begin. The author hopes to be able to examine representative

British and other soils from this point of view, and is of opinion that it will prove to be more valuable as a foundation for soil classification than the extreme climatic view which was the basis of the Russian work, or the geological aspect which, while of much value in parts of this country, breaks down badly in others. As will be seen, the resolution of the profile into its constituents and an examination of these in the light of soil and atmospheric climate in reality constitute a combination of both methods of approach.

The purpose of this short contribution is to draw attention to the composite character of the soil profiles, and to the importance of considering the interrelation of these, and to emphasize anew the part played by soil climate as distinct from atmospheric climate in soil development.

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PLASTOMETRIC STUDIES OF SOIL AND CLAY PASTES.

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(With Six Text-figures.)

Introduction.

The colloidal properties of the soil are now generally recognised as exercising a highly important contribution to its physical and physico-chemical characteristics. In the laboratory weak suspensions of the finer soil particles show a complicated range of colloidal properties, which are as yet incompletely understood. Nevertheless, the phenomenon of flocculation of such suspensions and the aggregation into compound particles characterising good tilth in field soils are two processes so strikingly analogous that the phenomena are considered to be closely related. Although attempts have been made to explain this relationship in exact terms it still resists solution. Evidently it is not sufficient to make direct comparison between such extreme conditions as flocculation in weak suspensions and the behaviour of soil in the field; a more comprehensive attack is needed in which the changing properties of a soil-water mixture are studied while the concentration of the soil is progressively diminished from that in the field to the weak suspensions (*circa* 1–2 per cent.) employed in studies of flocculation, cataphoresis, etc.

This investigation is now in progress in the Soil Physics Department at Rothamsted. It comprises a continuation of the field studies of cultivation processes and dynamometer measurements (1, 2), and laboratory work over the whole range of moisture content mentioned above. The latter may for convenience be divided into three ranges: the first in which the moisture content is of the same order as that in the field, the second in which the material is in the form of thick or semi-fluid pastes, and the third, or soil suspension range, in which the soil concentration rarely exceeds 5 per cent. by weight. In the first range, if the soil is well worked up with water a mass is obtained in which cohesive properties are paramount. The general behaviour over this stage is already known in its essentials (3). Over the intermediate range the paste displays both solid and fluid (or viscous) properties, while in the weak concentrations constituting the third range, this duality is shown by the

contrasting hydrophobe and hydrophyl properties of the colloidal system.

The present paper deals only with the intermediate range, and gives an account of some important relationships between the experimental results and other measurements made under very different conditions.

The experimental method adopted is to force pastes through capillaries of known dimensions under accurately measurable stresses and strain-conditions. As the range of viscous and solid properties displayed by materials under stress is of great importance in many industries, this experimental method has been much used, and the general descriptive term "plastometry" is applied to it. Much of the published work can be regarded as empirical as it is based either on defective experimental methods, or on an incomplete theory.

For this reason, and also because the plastometric study of soil has not hitherto received attention in soil studies, a brief statement will be given here of the theoretical basis together with a full description of the present experimental method and possible extensions of the technique.

THEORY OF THE METHOD.

The flow of *fluids* through capillary tubes is expressed by the classical equation of Poiseuille, which states that the quantity flowing in unit time, V, under an effective pressure, P, through a tube of length L and radius R is

$$V = \pi P R^4 / 8 L \eta \qquad \text{......(1)},$$

the quantity η being the viscosity of the fluid.

In the case of liquids containing particles in suspension, and of pastes in which the relation between the two phases is of a more intimate character, the Poiseuille relationship no longer holds. Experimental work already published from this laboratory (4) and further results (5) show that the flow of a soil or clay paste through capillary tubes systematically changes its character as the pressure applied is increased. Considering first the case of a single capillary tube, four stages can be distinguished:

Stage 1. The paste does not begin to flow until a certain pressure has been reached. Below this critical pressure therefore the mixture is behaving as a solid.

Stage 2. The paste moves as a rigid cylindrical plug through a thin envelope of the fluid, which adheres both to the wall of the tube and to the plug. Over this range the volume of flow in unit time increases linearly with the pressure applied.

Stage 3. The diameter of the plug decreases. In the annular region surrounding the plug, the paste has a stream-line flow. The general motion can be pictured as similar to the drawing out of a telescope, the eyepiece tube representing the central plug and the remaining tubes the stream-line shells surrounding it. The volume of flow therefore increases at a faster rate than the pressure applied. As the pressure is increased further the diameter of the central plug progressively decreases. Over this region the bulk of the paste has a stream-line motion, with the result that the relation between volume of flow and applied pressure rapidly approaches linearity.

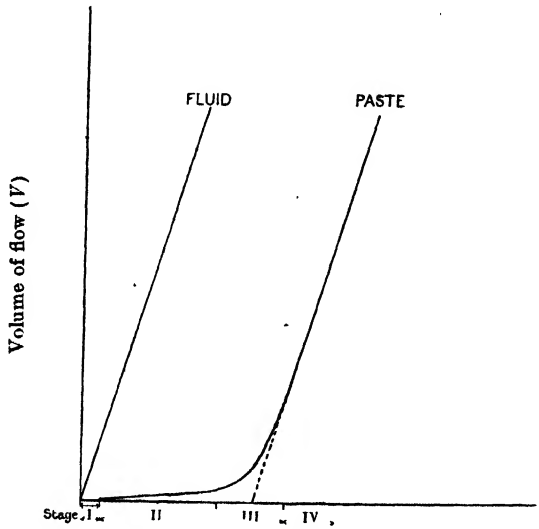

Fig. 1.

Stage 4. Over this region the flow is so nearly stream-line throughout that the error involved in assuring linearity between volume of flow and pressure becomes negligible.

Hence when the volume of flow is plotted against pressure applied, a curve of the type shown diagrammatically in Fig. 1 is obtained. Actually a complete experimental curve shows such a wide range of rates of flow that, on a vertical scale suitable for Stages 3 and 4, the earlier stages would be hardly appreciable. The flow in the later stages is about one hundred times as fast as at the beginning.

Evidently the behaviour of the paste in general differs strikingly from that of a true liquid, for equation (1) shows that for a given capillary

the relation between the volume flow V and pressure P for a fluid is represented (as in Fig. 1) by a straight line passing through the origin. But as Stage 4 in the curve for paste is sensibly straight and constitutes the greater part of a complete experimental curve, there is evidently some similarity between the behaviour of a paste over this range, and that of a true liquid.

For the purpose of examining this behaviour more conveniently it is usual to plot the results in a slightly different manner from that used in Fig. 1. The equation for a fluid (equation 1) can be written:

$$V/\pi R^3 = (PR/2L)\,(1/4\eta) \qquad \ldots\ldots(2).$$

Hence if a number of different sized capillary tubes were used in turn, and a series of measurements of V for different values of P were made on each tube, the curve obtained on plotting $V/\pi R^3$ as ordinates, and $PR/2L$ as abscissae, would be a single straight line passing through the origin as shown in Fig. 2.

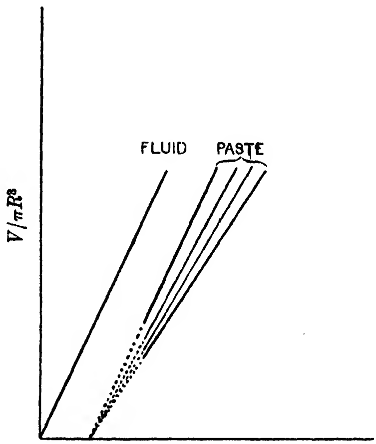

Fig. 2.

The slope of the curve is evidently $1/4\eta$, *i.e.* a numerical constant (1/4) multiplied by the inverse of the viscosity η. The inverse viscosity is usually called the fluidity. If the results had been plotted on the basis of Fig. 1, a family of straight lines would have been obtained, the slope of each depending both on the fluidity of the liquid and on the dimensions of the capillary. The method adopted in Fig. 2, however, is not only more convenient graphically, but has a definite physical significance. For the pressure P is, by definition, a force per unit area; the total force applied across the face of the capillary of radius R is thus $\pi R^2 P$. In the steady state of flow this pressure is balanced by an equal and opposite force, namely the total stress round the walls of the capillary, whose area is $2\pi RL$. Hence the stress per unit area of the wall is $\pi R^2 P/2\pi RL$, or $PR/2L$. Thus the abscissae in Fig. 2 represent stresses per unit area irrespective of the capillary dimensions. Further, the slope of the curve for a true fluid represents one-fourth of the fluidity. When the same

method of plotting is applied to the results for a *paste* (attention being confined to Stage 4) it is found as a general rule that a single straight line is not obtained. Instead a family of straight lines is given, one for each capillary used. It is found as an experimental fact, for all clay and soil pastes so far investigated, that when the straight line portion (Stage 4) of each curve is extrapolated to the stress axis, they all meet at a point. A series of such curves is shown diagrammatically in Fig. 2. Two deductions can be made: first, that there is a certain definite shearing stress for the material represented by the common intercept of the extrapolated curves on the stress axis, which, if subtracted from the total applied stress, then enables the graph *for any one capillary* to be treated as analogous to the single graph that completely represents the behaviour of a true fluid (geometrically this is equivalent to shifting the origin along the axis of stress to the point of intersection); secondly, the fact that a family of curves is obtained with a paste indicates that the fluidity measurement (the slope of the curve) is not independent of the dimensions of the capillary. The theory accounting for this departure and the method of treating the results to obtain a measure of the fluidity is dealt with later.

This definite shearing stress is usually called the "static rigidity" of the system. It represents the energy required just to cause the paste to flow, and is a measure of the solid cohesive properties of the system. Objection has been raised to this conception on the grounds that the intersection with the stress axis, being obtained by extrapolation of an asymptotic portion of the curve, lies outside that curve, and has therefore no physical significance (6). On the other hand, it appears valid to assume that over Stage 4 of the curve the energy represented by the "static rigidity" is used up in overcoming the friction between solid particles of the paste, and the effective stress causing flow is therefore less than the total stress by a constant amount.

No equation has yet been formulated that completely defines the relationships between volume of flow per second and applied pressure outlined above. Buckingham (7) put forward one consisting essentially of two terms, one of which covers Stage 2 (solid plug flow) and the other Stages 3 and 4 (partial to complete stream-line flow). The fact that in general a series of curves is obtained for different capillaries when $V/\pi R^3$ is plotted against $PR/2L$ (see Fig. 2) shows that Buckingham's equation does not directly apply to soil and clay pastes. But so far as flow as a solid plug is concerned, earlier work in this laboratory (4) has shown that the following equation (which is a

slightly modified form of Buckingham's first term to include Stage 1) is satisfactory:

$$V = \frac{\pi R^3 \epsilon\phi\,(P - a)}{2L} \qquad \ldots\ldots(3),$$

where ϵ is thickness of water envelope through which the plug is flowing, ϕ is its fluidity (not necessarily that of water in bulk), a is the pressure attained when the plug just begins to move and V, R, P and L have the meanings already given. But even this equation does not hold invariably; if, instead of using a paste made from moist soil fresh from the field, the soil is air-dried before use, the simple plug motion of Stage 2 does not generally occur, and this part of the volume-pressure curve is no longer a straight line.

This effect is almost certainly associated with the colloidal properties of the soil, but the exact mechanism of the process is not yet understood. The change is certainly a reversible one, because pastes made from moist surface soil do not show this abnormal behaviour, although the soil frequently passes through the air-dry condition in the field. Nevertheless, the process can hardly be a *simple* reversible one, because there is a wide variation in the ease with which air-dried samples of soil recover the property of giving a simple plug flow of the type defined by equation (3). The phenomenon requires further elucidation. Inspection of equation (3) shows that the properties defined by the constants ϵ, ϕ, and a, may be affected by the air-drying, since the other symbols refer only to the dimensions of the capillary and to the recorded experimental measurements. Experimental work has shown that the values of a and of the product $\epsilon\phi$ are the same in a "recovered" soil and in the original soil before air-drying.

With regard to the third stage of plastic flow, no satisfactory equation is yet available. Reiner (8) and later Buckingham (7) independently derived equations based on the conception that the plug type of flow was gradually replaced by stream-line flow. Although this conception is known to be qualitatively correct the data obtained in this laboratory do not conform completely to the equations of Reiner and Buckingham.

In the fourth or stream-line stage, as already pointed out, the divergence of the curves connecting $V/\pi R^3$ and $PR/2L$ for different capillaries (see Fig. 2) precludes the possibility of directly obtaining a simple viscous constant independent of capillary dimensions. Considerable attention has been devoted to this point, and a full account is given elsewhere (5); in the present paper only a brief outline will be given. It has already been shown (see Fig. 2 and discussion thereon) that over

the fourth region of plastic flow it is necessary in any case to modify the Poiseuille equation by subtracting from the pressure (P) an amount equivalent to the static rigidity, so that equation (1) becomes

$$V = \frac{\pi R^4 (P - c)}{8L\eta'} \qquad \text{......(4).}$$

The quantity η' corresponds to the viscosity η of a true fluid, and is called the "pseudo-viscosity."

If this modified equation be applicable to the fourth stage of flow, then on plotting $V/\pi R^3$ against $PR/2L$, as in Fig. 2, the points should all be on one straight line irrespective of the radii of the capillary tubes used, while actually it is found that a separate straight line is given for each capillary. To account for this divergence it is assumed that the properties of the paste become modified in the immediate neighbourhood of the tube wall, so that the rest of the paste has a different mean velocity from that to be expected from the equation (4). The experimentally measured total volume of flow (V) is therefore made up of two quantities, one of which obeys equation (4) while the other does not; the hypothesis given above enables the experimental results to be interpreted by eliminating the latter. Equation (4) can be written

$$\frac{V/\pi R^2}{(P - c)\, R/2L} = \frac{R}{4\eta'}. \qquad \text{......(5).}$$

The left-hand side of this equation is evidently the mean velocity of flow for the whole paste ($V/\pi R^2$), over the effective stress $(P - c)\, R/2L$ at the wall of the tube. We can now modify this equation by subtracting the quantity σ_0, which represents the contribution of the modified layer at the wall:

$$\frac{V/\pi R^2}{(P - c)\, R/2L} - \sigma_0 = R/4\eta' \qquad \text{......(6).}$$

To apply this equation, the slope of velocity-stress curve

$$\left[\frac{V/\pi R^2}{(P - c)\, R/2L}\right]$$

for each tube is plotted against the radius of that tube. It is found that the points for the different capillaries fall on a straight line, which on extrapolation gives intercept σ_0 on the slope axis. This intercept is a measure of the extent of the anomalous behaviour near the wall, while the slope of the curve, as is also evident from equation (6), is equal to $1/4\eta'$, where η' is the pseudo-viscosity of the bulk of the system.

This equation is found to hold very well over a wide range of concentration and materials, subject to the restrictions that the capillary

diameter must not be so small that the modified layer forms an appreciable portion of the total flow, nor so large that stream-line motion is replaced by turbulent flow.

It is clear that the actual values of the plastic constants dealt with in the above discussion will depend on the concentration of paste used. In comparing different soils it is necessary to work either at the same concentration of paste, or to use each material at a number of different concentrations.

It will be realised from the above account of the theory of flow of plastic pastes, that although it is now possible to evaluate several general constants representing the plastic behaviour, the subject is a complicated one, and there is still much work to be done on the dynamics of the flow.

EXPERIMENTAL METHODS.

Arrangement of apparatus. The plastometer bulbs were made from two pipettes by bending round and cutting off the ends, the surfaces being ground flat (see Fig. 3). The volume of the bulbs used depends on

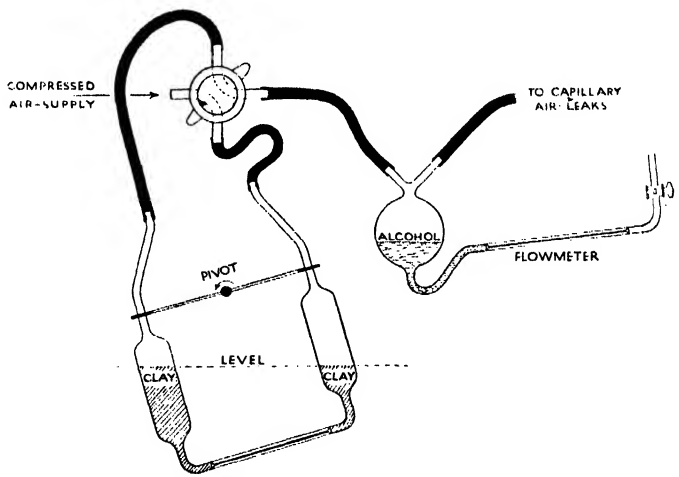

Fig. 3. Diagram of plastometer.

the range of stress to be investigated. Convenient sizes are 10, 50 and 100 c.c. each. The capillary tube is connected between them by means of short lengths of rubber tubing, the ends of the capillaries being ground to fit against the ends of the bulbs. The whole system is mounted on a stand which can be rotated on a pivot, so that when the paste is forced alternately from one bulb to the other, the surface of the paste in the bulbs

can be kept roughly in one and the same horizontal plane. This is effected by a chain which is fastened to the two ends of the stand, and passes over a toothed wheel above the apparatus, connected by means of a pulley system to a level-control wheel turned by hand.

The top ends of the bulbs, which are bent towards each other slightly for convenience, are connected by means of flexible rubber tubing to a four-way tap so that each can be connected alternatively to the air-pressure supply and the flowmeter. In the first shearing, one bulb is connected to the air-pressure supply, and the other to the flowmeter; in the second shearing the connections are reversed, and so on.

Compressed air is supplied from a foot pump and is stored at a pressure marked by a rough manometer, in a large stone bottle as a reservoir. It is released from this through a sensitive valve into a second reservoir (in order to insure a perfectly steady application of pressure) which is connected to the plastometer bulb. The second reservoir also connects with (1) a mercury manometer to measure higher pressures, (2) a water manometer for use at low pressure, (3) an exhaust to release pressure suddenly, and (4) a capillary leak to lower pressure slowly and steadily if required.

The paste can then be forced through the capillary at a series of different pressures which can be applied steadily, and measured accurately.

In order to measure the volume of flow per second, the air displaced by the flowing mass of paste is caused to pass through a flowmeter as shown in the figure, before escaping through one of a series of long capillary tubes. The flowmeter consists of a sensitive alcohol manometer set at a small angle to measure the pressure at the entrance to the air capillary. Since air is a true fluid, this pressure is proportional to the volume of air passing through the air-leak capillary per second, and hence to the flow per second of the paste in the plastometer.

It is necessary to adjust the zero of the flowmeter manometer before each run. When this has been done, the paste is forced through at a series of different pressures, and for each, the reading on the flowmeter is recorded. To convert this into c.c./sec. a factor is used depending on the dimensions of the particular capillary air-leak in use. To determine the factors, a glycerine-water mixture of convenient viscosity is passed through the apparatus, and the time required for it to flow through a bulb of known volume is recorded on a stop watch. This is repeated at different readings on the flowmeter, the reading being held constant during each run by keeping the level suitably adjusted.

In this way, a calibration curve for the flowmeter is obtained for each capillary air-leak.

The capillary and bulbs, the flowmeter, and the capillary air-leak are all kept in a thermostat at 25° C.

Plastometer capillaries. These are cut from the most uniform capillary tubes obtainable, and are carefully ground at the ends. The length is measured directly, and the radius is found by weighing the amount of mercury which will just fill the capillaries at a known temperature. This has been found more accurate than a determination by a viscometric method. Capillaries of very widely different lengths and radii have been used, but for ordinary purposes about 12 cm. is found a convenient length, with a radius of from 0·04 to about 0·12 cm.

Preparation of materials. It has not been found possible to get satisfactory results with any paste containing solid particles which will not pass through a sieve with 100 mesh per inch. Soils, whether direct from the field, or air-dried, are made up into a paste with distilled water and forced through such a sieve, the coarse sand remaining on the sieve being discarded.

For this reason the plastometer is not suited to the study of soil containing a high percentage of coarse sand. It is also necessary to pass pastes prepared from clay fractions through sieves, since the presence of a single particle of grit would vitiate the experiment.

The moisture content of the sieved paste is then determined by heating a sample in an oven at 160° C. for one hour. (This gives results closely correlable with those obtained in a steam oven for 24 hours.) In preparing a series of pastes from soils direct from the field at the same concentration in the paste, it is found unsatisfactory to determine the moisture contents of the original soils and then to add the required amounts of water to form the pastes, since soils do not take up the water evenly. The much more laborious process of drying down the soils gently in the laboratory until each attains a given moisture content and then adding the same quantity of water to each is best employed.

The paste is brought up to temperature by immersion in a bottle in the thermostat, and then sucked into the bulbs, and the apparatus is connected up, a capillary of suitable dimensions being used. It is best to get curves for each sample with at least 4 or 5 capillary tubes of different dimensions. The paste should be transferred to a bottle and shaken between runs with the different capillaries. Before any readings are taken, the paste is always sheared once through the capillary. This not only enables the operator to decide on the best series of pressures

to apply, but removes any irregularities still present in the paste, and eliminates the phenomenon known as thixotropic structure.

The flowmeter readings for a series of constant pressures are then recorded, the number of separate applications of pressure during each shear depending on the rate of shear used, and the dimensions of the bulbs. For accurate work, the bulbs are finally connected to each other without a capillary and a curve is obtained giving the amount of pressure taken up by the bulbs themselves for any given volume of flow per sec. This is subtracted from the observed pressure before calculating the stresses.

Since the range of the flowmeter for a given air-leak is small, it is best, if a wide range of stresses is to be investigated, to use at least two different air-leaks during each run.

Although this process sounds somewhat laborious it has been found possible to take the measurements quite quickly. Thus, to determine the plastometric constants of a given paste at a single concentration even with five separate capillaries would involve only about 1½ hours experimentation and an hour's calculation. If the operator is sure that he is working on the straight-line portion of the curves, much time can be saved by applying only three different pressures for each capillary and allowing the whole of the paste to flow across for each reading—taking a mean flowmeter reading for the whole run. In this way quite satisfactory constants have been obtained for ordinary purposes necessitating only three calculations of stress and mean velocity for each tube.

The above apparatus and technique, although considerably modified for soil work, are essentially those of Bingham (9), who has applied a similar method to the study of paints, etc.

Experimental results.

Work is still in progress on the relation between the pseudo-viscosity of soil and clay pastes and other physical properties of the soil, and will form the subject of a later paper. The present account will be confined to certain relations found to exist between the static rigidity and various field characteristics of soil. The full meaning of these results have still to be worked out; their immediate importance lies in the demonstration that the constants determined by plastometric measurements in the laboratory are related to field and laboratory measurements made under very different experimental conditions.

Static rigidity and dynamometer measurements. Numerous measurements with a dynamometer of the resistance offered by soils to the

passage of cultivation implements have been made at Rothamsted (1, 2) and the results have been shown to represent a characteristic property of the soil. Considerable variations in the resistance may be found from point to point over a given level area, and these variations bear no obvious relationships to such factors as moisture content, percentage of clay, etc. Nevertheless, the close concordance between successive results even over a series of years, unquestionably shows that the dynamometer measurement represents some definite soil property. It can conveniently be regarded as an integrated measure of the various physical properties such as cohesion and plasticity (using these terms in their general meaning) that are brought into play when the soil mass is cut by the coulter and share and turned over by the mouldboard.

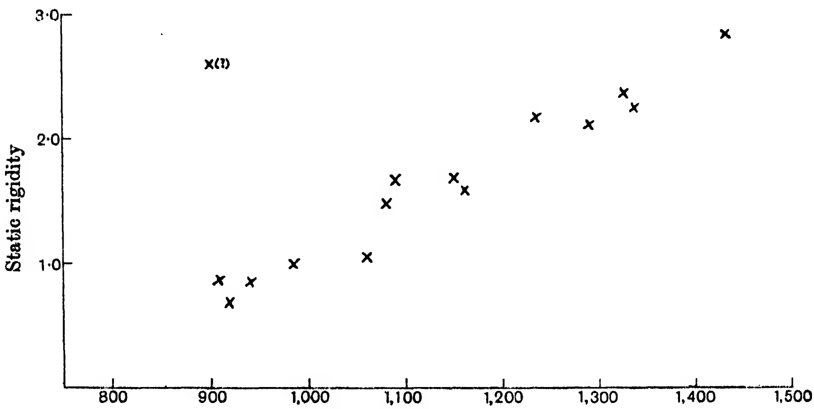

Fig. 4. Static rigidity and soil resistance, Broadbalk field.

Samples of soil were taken from definite parts of various fields for which full dynamometer records exist, and made into pastes of the same concentration as described above.

The relation between the static rigidity of the paste and the dynamometer measurement for that part of the field from which the particular sample was taken is shown for Broadbalk field in Fig. 4. It is evident that a close correlation exists. The result of a similar experiment for Sawyers field are given in Table I. The correlation coefficient is again significant, but the results are not so striking as those for Broadbalk. Table I also shows the values for the light sandy soil at Woburn, and here the relation is not so significant. This may indicate that it does not hold for sandy soils, although it should be remembered that the coarse

sand is removed before plastometric measurements can be made, and as the Woburn soil contains some 50 per cent. coarse sand it is perhaps not surprising that the results show so little regularity.

Table I. *Dynamometer measurements and static rigidity.*

1. Sawyers field, Rothamsted, air-dry soil			2. Stackyard field (permanent barley plots), Woburn, air-dry soil		
Plots	Dynamometer measurements	Static rigidity	Plot	Dynamometer measurements	Static rigidity
H 1	1220	1·98	5 BX	214	1·46
A 1	1235	1·96	2 AA	225	2·00
H 2	1244	2·12	8 BB	230	1·94
F 1	1280	2·14	9 B	235	2·40
B 3	1280	2·16	8 A	238	1·18
G 3	1306	2·18	10 A	253	2·80
G 4	1350	2·51	2 BB	255	3·33
C 1	1350	2·14	10 B (top)	287	1·50
H 4	1360	2·44	11 A	305	2·47
C 5	1410 (?)	2·07			
E 4	1520	2·77			

There is one point on the Broadbalk curve which falls very far from the general line. This refers to the sample taken from the plot which has received heavy annual dressings of dung for the past 80 years. The pseudo-viscosity was very high, and an exact determination of static rigidity was difficult; nevertheless, it may well constitute a real exception to the relationship which, as is shown also in Table I, is not necessarily of general application.

The effect of chalk, lime and cyanamide. Dynamometer measurements, made several years after the application of heavy dressings of chalk, have shown that the soil resistance is appreciably reduced as compared with untreated soil. Experiments with smaller dressings of chalk and equivalent dressings of lime have shown no appreciable reduction in soil resistance, at any rate in the year following the application. It is of interest to see whether there is any effect on the static rigidity. A large sample of an acid surface soil adjacent to the Park grass plots, Rothamsted, was divided into a number of separate portions, to each of which was added a certain quantity of chalk, lime, or cyanamide, with thorough admixture. The amounts of lime, chalk, and cyanamide added to the soil were calculated on the basis that one part per thousand corresponded to 1 ton per acre in the field. After definite periods of time, a sample of each of these was removed, and made up into a paste at a moisture content of 50 per cent. in the usual way. The paste stood for 1 hour during the moisture content determination, and then, after the slight

necessary adjustment in concentration, was tested in the plastometer. The static rigidities are given in Table II. This table shows that:

(1) Large quantities of chalk reduce the static rigidity but take some days to effect the reduction.

(2) Large quantities of slaked lime likewise reduce the rigidity, but the effect is much more rapid.

(3) Quantities of chalk and lime too small to affect the dynamometer measurements appreciably reduce the static rigidity.

Table II. *Effect of chalk, lime and cyanamide in various quantities on static rigidity of* 50 *per cent. paste, Park grass surface soil.*

Sample	Treatment	Duration (days)	Static rigidity (C)
K	Control	—	1·43
L	50 tons chalk p.a.	0	1·43
		7	1·18
		25	0·65
M	38 tons slaked lime p.a.	0	0·47
		7	0·15
		25	0·19
N	10 tons slaked lime p.a.	0	1·12
		11	0·77*
		26	0·86
O	2 tons cyanamide p.a.	0	1·41
		10	0·80
		24	0·56
P	1 ton slaked lime p.a.	0	1·39
		9	0·86
		26	0·94*
Q	½ ton cyanamide p.a.	1	1·30
		27	0·55

* Results known to be somewhat uncertain.

Static rigidity and concentration of paste. The importance of the concentration of soil in the paste was referred to at the end of the section dealing with the theory of the method, for it is clear that this factor must be taken into account in all measurements. A series of experiments was made therefore on the relation between concentration of paste and static rigidity, for a wide range of soils and clays. The results are shown in Fig. 5. The relation is evidently complex but the relative position of the curves on the diagram gives a clear indication of the extent to which colloidal properties are shown by the various materials. The curves for bentonite and the clay fractions are at the left-hand end; in other words, these materials display static rigidity at low concentration, whereas the lighter soils (at the right-hand end of the diagram) must be used in much higher concentrations before measurable static rigidity occurs.

Static rigidity and "sticky point." Recent work by Keen and Coutts on a wide variety of soils (10), and by Coutts (11) on Natal soils, has shown that the moisture content at which a kneaded and plastic mass of soil is about to become sticky is a well-defined quantity that promises to be of service as a "single-value" soil measurement. Although there are exceptions, in general the sticky point appears to give a fair measure of the extent to which colloidal properties of the soil are developed. The

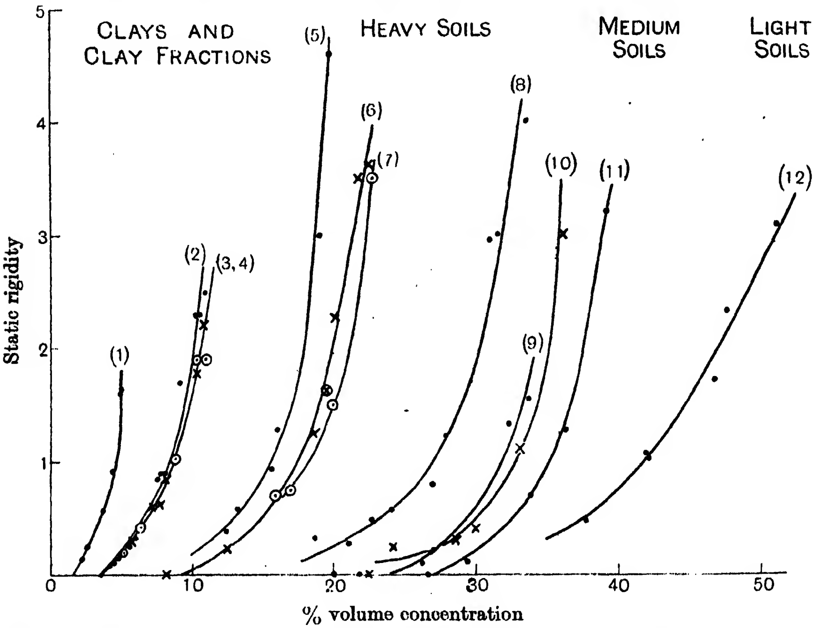

Fig. 5. Variation of static rigidity in concentration of material. (1) Natural bentonite. (2) Clay fraction, Broadbalk field. (3) Oolitic clay. (4) Liassic clay. (5) Fiji lateritic soil. (6) Natural kaolin. (7) Gold Coast soil. (8) Surface soil from park grass field (Rothamsted). (9) Broadbalk soil, farmyard manure plot. (10) Broadbalk soil, unmanured plot. (11) Beaumont alluvial subsoil. (12) Punjab soil.

results given in the preceding paragraph also suggest that the static rigidity-concentration curve may show a similar relationship. Inspection of Fig. 5 indicates that in the present stage of development of plastometric studies some convention must be adopted to select a single value of the static rigidity characterising a given soil. The most logical course would be to select the concentration corresponding to zero static rigidity, but the experimental error over this region becomes important, while extrapolation of the curve to the concentration axis is also subject to error. It appears better to adopt interpolation and to select that con-

centration at which any given material shows a static rigidity of a convenient constant value, *e.g.* one unit. This interpolation gives the moisture content at which the soil shows a definite degree of rigidity and is therefore analogous to the sticky point, differing only in the degree of rigidity chosen and in the method of measuring it. Among the small number of soils so far investigated, a close correlation exists between the two factors, except in the case of materials that are known to give anomalous behaviour in one or other of the measurements. Data for four normal soils are given in Fig. 6.

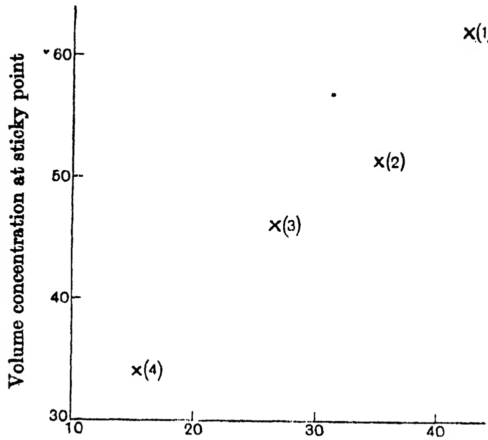

Fig. 6. (1) Punjab soil. (2) Beaumont alluvial subsoil. (3) Park grass, Rothamsted. (4) Fiji lateritic soil.

SUMMARY.

The laboratory study of the physical properties of soil and clays can conveniently be divided into three stages:

(*a*) Moisture content comparable to that under field conditions.

(*b*) Thick pastes.

(*c*) Weak suspensions.

The use of the plastometer for experimental work on intermediate stage is described and recent developments of the theory of the flow for thick pastes under stress are outlined. It is shown that certain constants defining the material can be obtained from the experimental data. The two to which special attention is given are the pseudo-viscosity (a quantity analogous to the viscosity of true fluids) and the static

rigidity (which represents the energy required just to cause the paste to flow and a measure of the solid cohesive properties of the system). The latter quantity is related to other physical measurements made under very different experimental conditions, *e.g.* the resistance of the soil to the passage of cultivation implements; the effect of chalk, etc., on the soil resistance; the moisture content at which a well-kneaded mass of soil is about to become sticky.

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SUGAR-BEET PULP AS A SOURCE OF PECTIN.

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Introduction.

With the growth of the beet-sugar industry in this country, sugar-beet pulp is becoming available in greatly increasing amounts. Thus, during the season 1927–28, the total production of dried beet pulp amounted to 91,400 tons, as compared with 21,800 tons produced during the season 1925–26. Although during the early years of the sugar industry there was only a restricted demand for sugar-beet pulp for home-feeding, the recognition of the highly satisfactory nutritive properties of this by-product(1) has led to a much brisker demand on the part of stock-feeders in this country and, as a consequence, demand has overtaken supply. It does not seem likely at present, therefore, that there will be any necessity for laying down any methods of utilising sugar-beet pulp beyond that of its direct usage as a food for farm stock.

With a view to possible future developments, however, the writers deemed it advisable to test the practicability of utilising sugar-beet pulp as a starting point for the manufacture of pectin. Dried sugar-beet pulp contains about 67 per cent. of N-free extractives (dry matter basis) and a large proportion of this constituent is known to be in the form of pectose. It appeared probable, therefore, that dried sugar-beet pulp would form a cheap and convenient source of pectin for use in the manufacture of jams and fruit jellies. A scheme of experiments was drawn up to enable information to be gained on the following questions: (1) What is the percentage of pectose in dried sugar-beet pulp? (2) What is the best method of extracting the pectin? (3) How does the jellying power of beet pectin compare with that of apple pectin?

I. Extraction of pectin from dried sugar-beet pulp.

In its insoluble form, pectin constitutes, in loose chemical association with cellulose, the middle lamella of the cell walls of many plant tissues, such as those of fleshy roots, stems and fruits like turnips, rhubarb stems, apples, cherries, etc. This insoluble form is known as pectose. The liberation of the soluble pectin from this insoluble pectin-cellulose complex is readily effected by hydrolysis with hot dilute acids, such as oxalic,

tartaric, citric and malic acids, $N/20$ HCl, etc. A 0·5 per cent. solution of ammonium oxalate at 100° C. has been shown to be an effective reagent for extracting the pectic substances of plant tissues (2), the extraction probably depending on the hydrolytic action of oxalic acid liberated by dissociation of the ammonium salt during the treatment. If this explanation be correct, it would be anticipated that a 0·5 per cent. solution of free oxalic acid, owing to its quicker hydrolytic action, would bring about a more rapid breakdown of pectose to pectin. This in fact has been found to be the case in the present investigation. When the tissue to be extracted contains free organic acid, the extraction of the pectin can be effected by means of water at 100° C. (or at 110° C. in an autoclave).

Extraction with 0·5 *per cent. ammonium oxalate.*

Exp. 1. After conducting an initial enquiry into the best conditions for the extraction, the following experiment was carried out. To 50 gm. of finely ground sugar-beet pulp was added 500 c.c. of 0·5 per cent. ammonium oxalate solution. The mixture was immersed in boiling water for 1 hour and was then removed and allowed to cool for 2 hours. The solvent was completely absorbed by the pulp. By squeezing through linen, 250 c.c. of extract was strained out. From this, on standing in a cylinder for some hours, a small amount of finely-divided discoloured material settled out, leaving an almost colourless supernatant liquid. 5 c.c. of this clear extract was now run slowly, with stirring, into 50 c.c. of 95 per cent. alcohol. After standing 30 minutes, the gelatinous white precipitate thus obtained was filtered on to a counterpoised filter paper, washed with a little alcohol, dried and weighed. The weight corresponded with a total extraction of 3·95 gm. of crude pectin.

To the residue from the foregoing extraction was now added 500 c.c. water and the mixture was digested for 1 hour in boiling water. After cooling and straining through linen, 430 c.c. of extract was obtained. This was concentrated to 100 c.c. on the water bath and a 5 c.c. portion was precipitated in 95 per cent. alcohol. The weight of precipitate obtained in this way corresponded with an extraction of 2·52 gm. crude pectin. The total weight of pectin from the two extractions, namely, 6·47 gm., was equal to 15·9 per cent. of the dry matter of the sugar-beet pulp. (Moisture content of pulp = 18·6 per cent.)

The purity of the alcohol-precipitated pectin from the first extraction was next investigated. 5 c.c. of clear extract was precipitated in 50 c.c. of 95 per cent. alcohol. The crude pectin was filtered off, washed with a

little alcohol, redissolved in a little water and re-precipitated by alcohol. The twice-precipitated pectin was then dissolved in water and its content of pure pectin determined by the calcium pectate method of Carré and Haynes (3). It was found that the crude alcohol-precipitated pectin contained about 60 per cent. of pure pectin.

It is clear, therefore, that the gelatinous precipitate obtained by running extracts of beet pulp into alcohol may not consist wholly of pectin. Nevertheless, for the following reasons, it was found convenient to employ this method of isolation in evaluating the strength of the beet pulp extracts: (1) The Carré and Haynes' method of estimating pectin is intricate and laborious, and in view of the disappointing results which were obtained in the investigation of the jellying power of beet pectin, it was not deemed worth while to lengthen out the investigation by adopting this method. (2) The alcohol method enables the efficiency of the different methods of extraction to be compared quickly and conveniently. (3) Commercial preparations of apple pectin never consist of pure pectin. Indeed, attempts to purify crude pectin by repeated precipitations in alcohol usually result in a lowering of the jellying power of the preparation.

Exp. 2. In order to find out how much crude pectin can be obtained from dried sugar-beet pulp by extraction with 0·5 per cent. ammonium oxalate, 50 gm. of finely ground beet pulp was submitted to the treatment described under Exp. 1. To the residue, after the first extraction, a further 250 c.c. of 0·5 per cent. ammonium oxalate was added and the process of extraction repeated. In this way, ten successive extractions were made. The liquid strained off after the eighth, ninth and tenth extractions was concentrated to half bulk on the water bath before precipitation with alcohol, in order to ensure quantitative separation of pectin. It should also be noted that from the fourth extraction onwards, the precipitate of crude pectin gradually lost the sticky, gelatinous nature[1] which characterised the samples obtained in the early extractions. The amounts of crude pectin obtained in the successive extractions are recorded in Table I.

The ten successive extractions with 0·5 per cent. ammonium oxalate yielded, on the basis of 100 gm. dried beet pulp, 46·3 gm. of crude pectin. The latter, however, contained some ammonium oxalate, which accounts for the figure 105 gm. in Table I B. 5 c.c. of 0·5 per cent. ammonium

[1] Drying of the gelatinous alcohol precipitate of pectin, either at the ordinary temperature in a vacuum desiccator or in the steam oven, caused it to pass over into a crisp, dark-coloured translucent mass.

oxalate, when run into 50 c.c. of 95 per cent. alcohol, gave a precipitate which, on drying, weighed 0·015 gm. Using this factor as a basis of correction, the yield of oxalate-free crude pectin amounted to 34·5 per cent. of the weight of dried sugar-beet pulp.

Table I. *Exhaustive extraction of beet pulp with* 0·5 *per cent. ammonium oxalate.*

A.

Extraction ...	1	2	3	4	5	6	7	8	9	10
Crude pectin as per cent. of dried beet pulp	7·35	9·28	7·07	4·67	3·85	3·65	2·88	2·74	2·70	2·11

B.

		gm.
Per 100 gm. of dried sugar-beet pulp	Dried residue after ten extractions ...	43·8
	Dry weight of sediments from extracts...	2·7
	Crude pectin extracted	46·3
	Moisture in original sugar-beet pulp ...	12·2
	Total	105·0

C.

	Contained in 100 gm. sugar-beet pulp before extraction gm.	Contained in dried residue after extraction gm.
Crude protein ...	8·77	7·23
Ether extract ...	1·11	0·34
N-free extractives ...	56·56	18·21
Crude fibre	17·28	15·84
Ash	4·08	2·18
Total	87·80	43·80

By comparing the composition of the beet pulp before and after extraction (Table I c), it is seen that ammonium oxalate solution mainly removes N-free extractives, rather more than 38 gm. of this constituent being taken out per 100 gm. beet pulp extracted. Minor amounts of crude protein, ether extract and ash were also dissolved out. The figures indicate a slight reduction of the fibre content as a consequence of the extraction. The fibre thus lost, however, is accounted for in terms of the small amounts of sediment which always settled out from the extracts on standing, such material being sufficiently finely divided to pass through the pores of the linen during the straining process.

Exp. 3. It was next decided to test whether a single prolonged extraction of beet pulp with 0·5 per cent. ammonium oxalate would achieve the same purpose as several successive short extractions. It seemed probable that this would be the case, since the amount of pectin extracted must depend on the extent to which the slow acid hydrolysis of the pectose is allowed to proceed. To 50 gm. of beet pulp in a flask

was added 500 c.c. of 0·5 per cent. ammonium oxalate and the mixture was digested for 30 hours in boiling water under a reflux condenser. After leaving to cool for 18 hours, the extract was strained off through a double thickness of linen. The volume of extract was 363 c.c. Its content of crude pectin was found, by the alcohol method, to be equal to 27·2 per cent. of the weight of sugar-beet pulp.

The beet pulp residue was next washed four times with distilled water, the liquid being strained off after each washing. The combined washings amounted to 536 c.c. 150 c.c. of this liquid was concentrated to 50 c.c. on the water bath and the crude pectin determined in 5 c.c. by precipitation in alcohol. The washings were found to contain crude pectin to an amount equal to 7·2 per cent. of the sugar-beet pulp.

Allowing for ammonium oxalate content, the single prolonged extraction gave a yield of crude pectin equal to 32·2 per cent. of the weight of beet pulp, compared with the 34·5 per cent. yield obtained by ten successive hourly extractions.

Extraction with 0·5 *per cent. oxalic acid.*

Exp. 1. 50 gm. of beet pulp was digested with 500 c.c. of 0·5 per cent. oxalic acid for 1 hour in boiling water. After cooling for 2 hours, the liquid was strained off through linen and its content of crude pectin determined by alcohol precipitation. The pulp residue was then submitted to two further extractions, in each case 250 c.c. of 0·5 per cent. oxalic acid being used.

Exp. 2. 50 gm. of beet pulp was digested for 26 hours at 100° C. with 500 c.c. of 0·5 per cent. oxalic acid. After cooling in the bath for 17 hours and straining off the extract, the residue was twice washed with measured amounts of distilled water, the liquid being pressed out each time.

The yields of crude pectin obtained in the oxalic acid extracts are compared in Table II with those obtained by extraction with ammonium oxalate. It is clear from Table II that 0·5 per cent. oxalic acid, owing to its stronger hydrolytic action, effects a much quicker extraction of pectin than does 0·5 per cent. ammonium oxalate. At the end of 1 hour, the oxalic acid had removed more than three times as much pectin as was obtained with ammonium oxalate. Indeed, the oxalic acid extract was so thick and viscid, that only by diluting with water could it be pressed through linen. The hydrolysis of pectose to pectin by 0·5 per cent. ammonium oxalate is comparatively slow, crude pectin being still

extracted after ten successive treatments of 1 hour each, whereas with 0·5 per cent. oxalic acid, the extraction was almost complete after two such treatments.

Table II. *Yields of crude pectin from sugar-beet pulp by extraction with* 0·5 *per cent. ammonium oxalate and with* 0·5 *per cent. oxalic acid (crude pectin expressed as per cent. of weight of dried sugar-beet pulp).*

	By successive hourly extractions		By single prolonged extraction	
Yield from	0·5 % amm. oxalate*	0·5 % oxalic acid	0·5 % amm. oxalate*	0·5 % oxalic acid
1st extraction	5·85	20·07	—	—
2nd extraction ...	7·76	7·16	—	—
3rd extraction ...	5·36	1·77	—	—
Remaining extractions	15·53	—	—	—
Total crude pectin (%)	34·50	29·00	32·20	23·70

* Yields corrected for the amm. oxalate contained in the alcohol precipitates of crude pectin.

There is, however, a further difference between the action of oxalic acid and of ammonium oxalate. The latter reagent, especially in the case of the single prolonged treatment, gave a better yield of crude pectin than was obtained with oxalic acid, despite the readier hydrolytic action of the free acid. This result is explained by the data recorded in Table III. It will be noted that with ammonium oxalate, after summating the moisture content of the beet pulp, the crude pectin extracted from it, the insoluble sediment and the dried residue, only 6·8 per cent. (ten successive short treatments) and 10 per cent. (single prolonged treatment) of the original beet pulp remained unaccounted for. When oxalic acid was used, however, the percentages of beet pulp unaccounted for were 21·0 per cent. (three successive short extractions) and 28·7 per cent. (single prolonged extraction) respectively. It would appear, therefore, that although the pectose of beet pulp is more readily hydrolysed to pectin by the use of the free oxalic acid, part of the crude pectin so formed is further acted on by the acid reagent with the production of a substance (or substances) which is not precipitated by alcohol. This inference was supported by the observation that the extract from the single prolonged digestion with oxalic acid reduced Fehling's solution quite copiously. In order to throw more light on this point, the following experiment was carried out.

Exp. 3. 35 c.c. of the extract obtained by prolonged digestion of beet pulp at 100° C. with 0·5 per cent. oxalic acid was precipitated in alcohol. The precipitate of pectin was filtered off, well washed with alcohol and

dried. It was then dissolved in 25 c.c. water, giving a solution which, unlike the original extract, displayed no reducing action towards Fehling's solution. To 12 c.c. of this solution was added 0·06 gm. of oxalic acid (0·5 per cent.) and the mixture was digested for 24 hours at 100° C. in a small bulb blown at the end of a length of glass tubing. The volume change of the reaction mixture under these conditions was only small. After allowing to cool, the liquid was restored to its exact original volume by the addition of a few drops of water.

The solution now reduced Fehling's solution copiously. 5 c.c. gave 0·068 gm. pectin on precipitation with alcohol, whereas 5 c.c. of the original solution contained 0·138 gm. pectin. During the digestion with oxalic acid, therefore, roughly half the alcohol-precipitable pectin must have undergone conversion into non-precipitable reducing material.

The difference between the action of ammonium oxalate and of oxalic acid on beet pulp is clear in the light of the foregoing experiment. Oxalic acid brings about a quick breakdown of pectose to pectin. During the extraction, however, the pectin so formed is undergoing a further slow hydrolysis to non-precipitable reducing material. This secondary hydrolytic action is much slower with ammonium oxalate, so that although the extraction of pectin is not so rapid with this reagent, the ultimate actual yield may be higher than with oxalic acid, an observation which agrees with the statement of earlier workers that 0·5 per cent. ammonium oxalate is an effective solvent for all the pectic substances of plant tissues.

Extraction with other reagents.

Further experiments were undertaken to test the efficiency of the process of extraction of pectin from dried sugar-beet pulp when 0·6 per cent. tartaric acid and $N/20$ hydrochloric acid were employed for the purpose. In both cases, the effect of a number of successive hourly extractions at 100° C. was compared with that obtained by a single extraction of 24 hours' duration. The results of these tests are shown in Table III.

The residue from the beet-pulp extractions was smallest when $N/20$ hydrochloric acid was employed. In this case, however, there was considerable secondary breakdown of pectin, a circumstance which naturally affected the yield of pectin. The data in Table III suggest that extraction with 0·6 per cent. tartaric acid may be expected to give a rather more satisfactory extraction of pectin than is obtained with 0·5 per cent. oxalic acid.

Table III. *Extraction of pectin from sugar-beet pulp by different reagents.*

	0·5 % amm. oxalate		0·5 % oxalic acid		0·6 % tartaric acid		$N/20$ hydrochloric acid	
	Successive short extractions	Single prolonged extraction	Successive short extractions	Single prolonged extraction	Successive short extractions	Single prolonged extraction	Successive short extractions	Single prolonged extraction
	%	%	%	%	%	%	%	%
Moisture in beet pulp ...	12·2	12·2	13·0	13·0	13·0	13·0	13·6	13·6
Crude pectin extracted ...	34·5	32·2	29·0	23·7	36·8	28·3	34·4	26·2
Insoluble sediment	2·7	2·9	4·5	2·0	3·5	1·9	4·9	2·0
Residue after extraction (dried)	43·8	42·7	32·5	32·6	32·1	32·7	24·1	29·9
Extracted from pulp but not precipitated by alcohol ...	6·8	10·0	21·0	28·7	14·6	24·1	23·0	28·3

Extraction with water alone.

Exp. 1. 50 gm. of dried sugar-beet pulp was extracted with 500 c.c. of boiling water for 24 hours under a reflux condenser. After cooling, the extract was strained off through linen and the residue was washed with cold water. The total alcohol-precipitable pectin in the extract and washings amounted to 31·6 per cent. of the original weight of beet pulp.

Exp. 2. A mixture of 50 gm. of dried sugar-beet pulp and 500 c.c. water was allowed to stand at room temperature for 5 days. After straining off the liquid, 200 c.c. was evaporated to 50 c.c. and the content of crude pectin determined by alcohol-precipitation. The amount extracted was equal to 3·2 per cent. of the weight of beet pulp.

II. Investigation ot jellying power of beet pectin.

Preparations of pectin, as obtained from fruits like apples, have considerable technical significance in the making of jams and fruit jellies. The property possessed by pectin of being able, when present in small amount and within a suitable range of pH, to impart a jelly condition to syrups of cane sugar has long been recognised. Certain features of this jellying reaction appear to have been definitely established[1]. A concentration of 0·2 to 1 per cent. of pectin would seem to be the optimum for jelly formation, the amounts of sugar and acid being adjusted to the concentration of pectin. At the same time, there is evidence that the amount of pectin may not always be the most important factor. Its physical condition and its chemical state (*i.e.* the degree of partial

[1] Much of the information in this paragraph is taken from Carré's "Chemistry of the Pectic Substances," which the writers had an opportunity of consulting in MS. form.

hydrolysis which it may have suffered during its extraction) may affect its capacity to produce a firm jelly. Attempts to purify pectin by repeated alcohol-precipitation also lead to a diminution of its jellying properties. Cane sugar is usually regarded as being the best for the production of jams and jellies, but there is now no doubt that beet sugar, in a proper degree of purity, is just as suitable. It has been shown that an excess of sugar may be responsible for failure to obtain a satisfactory consistency in the resultant jelly. A definite weight of pectin can apparently utilise a definite weight of sugar. A "tough" stringy consistency may result from the use of too little sugar, whereas an excess of sugar may give rise to a "soft" jelly or a syrup. With fruits naturally rich in pectin and acid, 1 to $1\frac{1}{2}$ parts of sugar should be added to 1 part of juice or whole fruit. Jellies are never formed in neutral media. Experiments with fruits of differing acidity indicate that the range of acidity for jelly formation is fairly wide, varying from 1·6 to 5·2 per cent. in terms of citric acid.

The results of numerous jellying trials carried out by Ogg[1] on the jellying power of cane sugar-apple pectin-acid mixtures led to the conclusion that optimum jellying occurs in an aqueous medium containing, per 100 gm., 65 gm. sugar, 0·2 gm. pectin and 1·25 gm. N/HCl.

The work carried out by the writers in connection with the jellying power of beet pectin falls naturally into three parts: (1) A large number of experiments was made in which the proportions of cane sugar, pectin and acid were varied. In parallel with these, similar trials were carried out in which the beet pectin was replaced by apple pectin. (2) Experiments were also devised to ascertain whether the unsuccessful results which were obtained in the first series of trials were to be ascribed to the influence of the mineral constituents of the beet-pectin preparations. In these experiments, beet pectin, in which the ash was replaced by the ash of apples, was employed. (3) Further experiments were made to find out if the lack of jellying power of the pectin from the dried sugar-beet pulp was connected with the influence of the high temperature to which the wet beet pulp is submitted during the factory drying process. For the purpose of these final experiments, pectin was extracted from fresh sugar beets.

Below are given the particulars of a series of trials in which viscous syrups were obtained, such syrups displaying no tendency to pass over into the form of jelly even after long standing. Since negative results

[1] From a personal communication by Dr W. G. Ogg, who also kindly furnished the writers with a sample of pectin prepared from apples.

were obtained in every trial with beet pectin, it would serve no purpose to cite the details of every single trial, and the following examples are merely given to indicate the lines on which the enquiry was conducted:

(1) In 100 gm.: 65 gm. cane sugar; 0·2 gm. pectin (water extracted and alcohol precipitated); 1·25 gm. $N/10$ HCl.

(2) In 100 gm.: 65 gm. cane sugar; 0·2 gm. pectin (tartaric acid extracted and alcohol precipitated); 1·25 gm. $N/10$ HCl.

Note. The mixtures (1) and (2) were made up in the proportions recommended by Ogg.

(3) In 100 gm.: 80 gm. cane sugar; 0·5 gm. pectin (tartaric acid extracted and alcohol precipitated); 5 gm. $N/10$ HCl.

(4) In 100 gm.: 80 gm. cane sugar; 0·5 gm. pectin (oxalic acid extracted and alcohol precipitated); 5 gm. $N/10$ HCl.

(5) In 100 gm.: 80 gm. cane sugar; 0·5 gm. pectin (amm. oxalate extracted and alcohol precipitated); 5 gm. $N/10$ HCl.

Note. Trials (1) to (5) were carried out without application of heat. In (3), (4) and (5), further pectin was added to bring up its amount to 1 per cent. No improvement was effected in this way, however. Under the conditions of (3), (4) and (5), apple pectin gave satisfactory jellies.

(6) In 100 gm.: 60 gm. cane sugar; 1·2 gm. pectin (tartaric acid extracted and alcohol precipitated); 1 gm. tartaric acid.

(7) In 100 gm.: 60 gm. cane sugar; 1·2 gm. pectin (water extracted and alcohol precipitated); 1 gm. tartaric acid.

Note. In (6) and (7) the mixture was first made up in water to 105 gm. and evaporated to 100 gm. on the steam bath. In both cases, a thick syrup was obtained, though apple pectin gave a good jelly under these conditions.

(8) Repetition of (7), replacing beet pectin by 3·6 gm. dried sugar-beet pulp. After digesting the mixture for half an hour on the steam bath, the liquor was strained off through muslin and evaporated for a short time on the steam bath. The thick syrup, even after days of standing, did not pass over into the condition of jelly.

(9) In experiments with apple pectin, it was discovered that readier jellying could be secured by using the tartaric acid extracts of apple pulp instead of solutions of alcohol-precipitated pectin. It seemed possible that the alcohol had the effect of slightly impairing the jellying power of the apple pectin. For this reason, experiments were made with beet pectin which had not been in contact with alcohol. Extracts were made by digesting sugar-beet pulp at 100° C. with 0·6 per cent. tartaric acid, these being brought to the necessary strength by concentration

in vacuo at low temperature. Negative results were again obtained, although the proportions of cane sugar, beet pectin and tartaric acid were varied considerably in the numerous trials which were carried out.

(10) If the effect of repeated alcohol-precipitation of apple pectin from its aqueous solution is to lower its jellying power, it is possible that this might be brought about by the removal in the alcohol of some essential constituent, the addition of which to mixtures of cane sugar, beet pectin and acid might lead to jellying. The alcoholic liquor from the precipitation of extracts of apple pectin was evaporated *in vacuo* at low temperature and varying amounts of the residue were added to mixtures of cane-sugar syrup, beet pectin and tartaric acid. In no case, however, were indications of jellying observed.

(11) Experiments were also made with pectin derived from dried sugar-beet pulp by extraction with 0·7 per cent. citric acid at 100° C. The extracts were not precipitated in alcohol, but were concentrated to the desired strength on the steam bath or *in vacuo* at a lower temperature. In making up the mixtures for jellying, the sugar and citric acid were maintained throughout at concentrations of 65 and 0·64 per cent. respectively, whereas the amount of pectin varied from 1 to 2 per cent. In some of the trials, the mixture was kept in closed vessels; in others, spontaneous evaporation in the open air was permitted, while in a third set, some of the material removed from apple pectin during alcohol-precipitation was added. In none of these cases was jellying, such as occurs with apple pectin, observed. Thick syrups were invariably obtained. These, if allowed to evaporate slowly in the air, became coated with a stiff, skin-like layer which gave the mixture the appearance of having "set." On breaking through this surface layer, however, the underlying mixture was found still to consist of a thick, viscous syrup.

The presence of malic acid in apples suggested the use of this acid instead of citric acid. Malic acid alone, as well as mixtures of malic and citric acids, was now employed for extracting the pectin from sugar-beet pulp and for making up the mixtures for further observations on jellying. These tests were no more successful than those already described. Thick syrups were obtained which, on long standing, displayed no tendency to develop the "lumpiness" of true jellies and the property which enables them to show clean, sharp edges when cut with a knife. In some of the trials, the sugar began to crystallise out after several days.

(12) The conditions in this test were such as to afford a very sharp contrast between the actions of apple pectin and beet pectin. To 60 gm. of cane sugar was added 0·93 gm. of alcohol-precipitated apple pectin.

The mixture was made up to 100 gm. by the addition of water. To a portion of this syrupy solution was stirred in tartaric acid equal to 0·75 per cent. of the weight of syrup. The latter changed *immediately* into a perfect and permanent jelly. An equally successful result was obtained by stirring in 0·66 per cent. of a mixture of malic and citric acids (5 : 1), the characteristic jelly being formed without delay. The original syrup of sugar and apple pectin, without acid, remained unchanged throughout a period of several weeks. During this time, the sugar displayed no tendency to crystallise out and the syrup kept its property of pouring freely. Neither did it lose its power to give a characteristic jelly as soon as a few crystals of tartaric acid were stirred into it.

For the purpose of comparison, a new sample of beet pectin was made by extracting well-washed sugar-beet pulp with 0·6 per cent. tartaric acid at 100° C., evaporating the extract to a small bulk at low temperature *in vacuo* and precipitating the pectin in alcohol. 100 gm. of a syrup containing 60 gm. of cane sugar and 0·93 gm. of beet pectin was then made up. To a portion of this syrup was stirred in 0·75 per cent. of tartaric acid. In direct contrast to the behaviour in the apple-pectin experiment just described, no jelly was formed. The addition of the acid seemed, as far as could be judged by the eye, to bring about a slight thickening of the medium, a change which was further accentuated on standing for several days. There was not the slightest resemblance, however, to the instantaneous jelly formation which characterised the tests in which apple pectin, instead of beet pectin, was employed.

(13) Further tests were made on the assumption that the jellying properties of any particular kind of pectin might be influenced by the nature and amount of the mineral constituents which it can be shown to contain even after repeated attempts to purify it by alcohol-precipitation. 50 gm. dried sugar-beet pulp was allowed to stand for 24 hours at the room temperature with 300 c.c. $N/10$ hydrochloric acid. It was then filtered off and washed thoroughly with water. This treatment removed about 61 per cent. of the lime in the sugar-beet pulp. The digestion with cold $N/10$ HCl was twice repeated and the residue was extracted in the usual way with 0·6 per cent. tartaric acid. The pectin, which was now almost ash-free, was isolated by alcohol-precipitation of the extract.

A number of tests carried out with this sample of pectin showed that the removal of the mineral constituents had led to no improvement of its jellying power. In further tests, the ash of apples was added in varying amounts to the jellying mixtures containing the nearly ash-free

beet pectin. Again the results were negative. In a final test, apple ash was added to the sugar-beet pulp after its treatment with cold $N/10$ HCl, the amount added being the same as would be contained in an equal weight of apple pulp. The pectin extracted under these conditions was also unable to impart a jelly-like character to suitable mixtures of acid and sugar syrup. It would appear, therefore, that the inability of beet pectin to occasion jellying is not connected with the nature or amount of its inorganic constituents, although the foregoing tests are perhaps not absolutely decisive on this point, since ignited ash was employed instead of the unchanged mineral substances.

(14) There still remained the possibility that the lack of jellying properties in beet pectin might be due to changes in the pectic material brought about during the drying at high temperature of the wet beet pulp in the factory, and that pectin from untreated sugar beets might resemble apple pectin in its power to form jellies with acid and sugar syrups. To test this possibility, a sample of fresh sugar beet was washed and pulped. The juice was expressed as completely as possible and the residue was exhaustively washed and pressed out with water. The final residue was extracted at 100° C. with 0·6 per cent. tartaric acid and the pectin, once precipitated in alcohol, was thoroughly tested for jellying properties. The results were no better than had been obtained for pectin extracted from dried sugar-beet pulp.

Summary.

It has been shown that dried sugar-beet pulp contains a high percentage of pectose. A number of successive digestions of 1 hour each with 0·5 per cent. ammonium oxalate at 100° C. extracts an amount of pectin equal to 34·5 per cent. of the weight of dried beet pulp, basing the determination on the weight of crude pectin precipitated when the extracts are run into 95 per cent. alcohol. A single prolonged digestion gives a yield of crude pectin equal to 32·2 per cent. of the dried beet pulp.

Digestion with acidic reagents, such as 0·5 per cent. oxalic acid, 0·6 per cent. tartaric acid, $N/20$ hydrochloric acid, etc., leads to a quicker extraction of pectin, owing to a speeding up of the pectose to pectin hydrolysis. The yield of pectin, however, is not thereby necessarily enhanced, since under such conditions the pectin undergoes a slow secondary hydrolysis during the extraction with the formation of reducing substances not precipitated by alcohol.

Prolonged digestion at 100° C. of dried sugar-beet pulp with water alone also leads to a satisfactory extraction of pectin.

Although beet-pulp pectin resembles the pectins from other sources in its general physical and chemical characteristics, it is sharply differentiated from apple pectin in its inability to impart a jelly condition to syrups containing suitable amounts of cane sugar and free acid. The reason for this absence of jellying power is not clear, though apparently it is not connected with the nature and amount of the mineral impurities in the pectin preparations. Nor is it to be ascribed to any changes in the character of the pectose of sugar-beet pulp during the factory process of drying the wet material.

The investigations described in this paper do not preclude the possibility of discovering a set of conditions under which the pectin extracted from sugar-beet pulp will display jellying properties similar to those of apple pectin. It is clear, however, that the extraction of pectin from beet pulp by methods similar to those employed for its preparation from apple pulp yields a product which, unlike apple pectin, has no technical significance in the processes of making jams and fruit jellies. It is to be concluded, therefore, that the proposals for utilising sugar-beet pulp in these industrial processes must, for the time being at any rate, be set aside.

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WATER LOSS AT WAD MEDANI. PART I.

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(With Three Text-figures.)

Long staple Egyptian cotton is the main crop of the Gezira Irrigation Scheme. The soil (1), (2) is alkaline and of low permeability, it contains in the upper four feet from 50 to 60 per cent. clay and about 0·2 per cent. soluble salts. The climate (3) is arid; at Wad Medani (14° 24′ N., 33° 31′ E.) the average annual rainfall is 400 mm., of which two-thirds is received in the months of July and August, and the mean annual temperature is 27·5° C. The conjunction of these adverse conditions imposes a severe strain on the cotton crop whose water requirement is correspondingly high. The investigations now in hand are ultimately directed to establishing relations between meteorological data and irrigation practice, but they will also be applied to physiological problems, *e.g.* to the task of ascertaining more precisely what amount of water is transpired by the plant and what is lost by evaporation from the soil. In the present communication an account is given of the method adopted for directly measuring water loss and of some preliminary experiments which indicate its scope.

I. Experimental procedure.

Changes in the total moisture content of soil can be estimated with reasonable accuracy by taking samples in sufficient numbers and to a sufficient depth. Information is required, however, as to the dependence of water loss on the moisture content of surface soil and experiments were accordingly made with shallow layers of soil. An attempt to measure the amount of water required to maintain these at different levels of moisture content had to be abandoned, but better results have now been obtained by exposing moist soil in aluminium boxes for short periods of time and under different conditions. Water loss from soil depends (1) on factors which affect a Piché evaporimeter, (2) on the nature of the soil, (3) on its moisture content, and (4) on other factors which do not affect a Piché evaporimeter. It will be shown below that a partial resolution along these lines is quite practicable, the general line of attack being to treat Piché figures as a separate problem and to explore the ways in which losses from soil are related to these.

The boxes (96 in number) are cylindrical, about 3 cm. deep and conveniently hold 100 gm. soil or 150 gm. sand, the area exposed to evaporation being 40·0 cm.^{2} Some days prior to exposure they are filled by small alternate additions of soil and water in such a manner as to provide four or eight batches of different make-up. The boxes are covered and allowed to stand so that the water may distribute itself through the solid medium and are subsequently divided into two similar groups of 48. These groups are exposed alternately for short periods, each box being weighed before and after exposure. As the experiment proceeds the range of moisture content diminishes and finally the boxes are placed in the oven and then weighed again. As the extent to which different factors affect the results is still to be ascertained, no attempt has been made to subject the present data to exhaustive analysis and the procedure adopted in condensing the observations is accordingly illustrated here. Loss from a single box is recorded as follows:

Before exposure	100 gm. soil	20·7 gm. water,
After exposure	100 gm. soil	19·3 gm. water,

Mean moisture content 20 per cent., loss 1·4 gm.

It is convenient to remember that as the area exposed was 40·0 cm.^{2} this loss corresponds to 1·4 imperial tons per acre and to 1·4 × 105/100 cubic metres per feddan. Mean moisture contents (M) and losses (L) are then tabulated and averaged as in the following table:

Table I. *Series* 5, *Period* (d).

	M	L	M	L	M	L	M	L
	5·2	0·3	10·8	2·0	24·6	3·7	39·3	5·3
	5·2	0·3	10·5	1·9	24·2	4·0	40·2	4·4
	5·2	0·3	10·2	1·9	25·1	3·8	39·0	5·5
	5·2	0·4	11·2	2·0	24·9	3·4	38·0	5·2
	5·2	0·3	10·6	2·0	24·9	3·1	38·5	5·0
	—	—	11·5	1·0	24·2	4·1	37·1	5·8
Mean ...	5·2	0·3	10·8	1·8	24·6	3·7	38·7	5·2

This table is a fair example of the extent of agreement obtained; the discrepancies are ascribed to differences in distribution of water within the boxes and to minor variations in external conditions.

An obvious objection to this method arises from the large number of weighings required. This difficulty is, however, overcome by use of a semi-self indicating fine balance (number A 534), supplied by Messrs W. and T. Avery, Ltd.

The capacity of this instrument is 500 gm.; it has a large and accessible weighing pan and carries an open scale from which fractions of the load less than 10 gm. are directly read. Owing, it is under-

stood, to regulations governing the construction of weighing machines for commercial use, facilities for adjustment are not ideal and we now await the arrival of two fittings designed to remedy this defect. As it stands, however, the instrument has been of great use in this investigation and is recommended for the consideration of soil workers.

II. DIURNAL VARIATION IN RATE OF WATER LOSS.

Boxes containing moistened Gezira soil or water were pressed flush with the surface of a bed of dry powdered soil, shaded from the sun by wooden boards, a foot above ground level, and exposed on April 29th–30th, 1929, for periods shown below:

Period (*a*)	6 a.m. to 8 a.m.	Period (*b*)	7.30 a.m. to 9.30 a.m.
(*c*)	9 a.m. to 11 a.m.	(*d*)	10.30 a.m. to 12.30 p.m.
(*e*)	12 noon to 2 p.m.	(*f*)	1.30 p.m. to 3.30 p.m.
(*g*)	3 p.m. to 5 p.m.	(*h*)	4.30 p.m. to 6.30 p.m.
(*i*)	6 p.m. to 8 p.m.	(*j*)	7.30 p.m. to 9.30 p.m.
(*k*)	9 p.m. to 6 a.m.		

The relation of losses (L) to mean moisture contents (M) observed in this series of exposures is shown below, the figures being means of six observations as described in the preceding section.

Table II.

	Soil		Soil		Soil		Soil		Water
Period	M	L	M	L	M	L	M	L	L
(*a*)	4·6	0·0	12·6	1·3	27·5	2·2	43·5	2·8	3·8
(*b*)	5·4	0·0	12·7	1·6	28·1	3·5	43·3	4·1	6·1
(*c*)	4·5	0·2	10·9	1·9	24·6	3·6	39·3	5·4	8·9
(*d*)	5·2	0·3	10·8	1·8	24·6	3·7	38·7	5·2	9·9
(*e*)	4·2	0·3	9·2	1·6	20·9	3·4	34·3	5·0	10·2
(*f*)	4·9	0·3	9·2	1·45	21·3	2·7	33·7	4·6	9·3
(*g*)	4·0	0·1	7·9	1·0	18·3	2·1	28·5	3·3	6·9
(*h*)	4·5	0·05	7·9	0·8	18·7	2·0	29·8	3·15	6·1
(*i*)	3·9	0·05	7·2	0·4	16·7	1·0	27·8	1·6	2·9
(*j*)	4·3	0·0	7·4	0·3	17·4	1·1	27·3	1·7	3·4
(*k*)	4·0	+0·4	6·5	0·9	14·4	3·5	24·0	6·0	15·1

By plotting these figures, losses for moisture contents of 5, 10, 15, 20, 25 and 30 per cent. were read. These readings multiplied by 0·75 give losses for consecutive periods of $1\frac{1}{2}$ hours instead of the original overlapping periods of 2 hours. To conform with this reduction the periods are numbered afresh in Table III and subsequently in the case of period (*k*) a small addition was made to represent the substitution of the 15 minutes, 6 a.m. to 6.15 a.m., for the 15 minutes 9 p.m. to

9.15 p.m. A loss estimated at 0·3 gm. occurred in the manipulation of tins containing water and allowance for this has been made in the following table which gives consecutive and total losses during the period of the experiment.

Table III.

Period	Time	% moisture content of soil 5	10	15	20	25	30	Water
1	6.15– 7·45	0·07	0·67	1·09	1·31	1·64	1·73	2·62
2	7.45– 9.15	+0·07	0·75	1·42	1·87	2·32	2·66	4·35
3	9.15–10.45	0·22	1·27	1·80	2·25	2·74	3·19	6·42
4	10.45–12.15	0·22	1·20	1·76	2·29	2·81	3·19	7·20
5	12.15– 1.45	0·37	1·35	1·87	2·44	2·93	3·37	7·42
6	1.45– 3.15	0·22	1·27	1·54	1·91	2·44	3·00	6·75
7	3.15– 4.45	0·22	0·90	1·31	1·73	2·17	2·59	4·95
8	4.45– 6.15	0·12	0·79	1·20	1·61	1·99	2·36	4·35
9	6.15– 7.45	0·10	0·45	0·67	0·89	1·09	1·31	1·95
10	7.45– 9.15	0·00	0·37	0·67	0·94	1·16	1·39	2·32
11	9.15– 6.15	0·01	2·00	3·65	4·95	6·25	7·45	14·80
—	—	0·01	0·09	0·14	0·17	0·20	0·23	0·26
Totals ...	24 hours	1·49	11·11	17·12	22·36	27·74	32·47	63·39

In Table IV the foregoing figures are expressed as percentages of the total loss for each column, while a final column gives the means for all moisture contents except the first (5 per cent. moisture). By reading horizontally across this table it is seen that percentage losses from soils having a moisture content of 10 per cent. or over and percentage losses from a free water surface are much alike. Moist soil and water thus show similar responses to changes in external conditions. The behaviour of soil of low moisture content is very different and is discussed in Section VI below.

Table IV. *Losses from soil and from water expressed as percentage of total loss in 24 hours.*

Period	% moisture content of soil 5	10	15	20	25	30	Water	Mean
1	4·70	6·02	6·37	5·85	5·90	5·32	4·13	5·60
2	+4·70	6·75	8·30	8·35	8·35	8·19	6·85	7·80
3	14·70	11·40	10·50	10·06	9·89	9·82	10·12	10·30
4	14·80	10·80	10·28	10·22	10·25	9·82	11·35	10·45
5	24·90	12·13	10·90	10·92	10·55	10·38	11·70	11·10
6	14·80	11·40	9·00	8·54	8·79	9·24	10·64	9·60
7	14·70	8·08	7·65	7·73	7·81	7·98	7·80	7·84
8	8·05	7·10	7·01	7·20	7·17	7·27	6·85	7·10
9	6·70	4·05	3·91	3·98	3·93	4·04	3·07	3·83
10	0·00	3·33	3·91	4·21	4·18	4·28	3·66	3·90
11	1·34	18·90	22·13	22·70	23·20	23·65	23·73	22·38

III. COMPARISON OF LOSSES FROM MOIST SOIL AND FROM WATER WITH READINGS OF A PICHÉ EVAPORIMETER.

(A) *Distribution of loss through the day.*

Readings of a Piché evaporimeter were made in conjunction with the experiment described in Section II. The tube, protected from the sun by a tin shield, was hung up with its evaporating disc near ground level and beside the shaded tins. The instrument showed surprising variations[1] in the rate of evaporation although these disappear when the observations are arranged to correspond with periods 1 to 11 of Table IV. Under the conditions of the experiment the total evaporation registered by the Piché tube was 27·00 mm. and in the following table the subdivisions of this amount have been expressed on a percentage basis for comparison with the mean losses which are taken from Table IV. Within periods 1 to 10 the two sets of figures have a correlation coefficient of + 0·95, but it will be observed that during the day the soil losses exceed Piché losses (both being expressed as percentages) and that during the night Piché losses exceed soil losses. Both sets of figures are related

Table V.

Period ...	1	2	3	4	5	6	7	8	9	10	11
Mean losses	5·60	7·80	10·30	10·45	11·10	9·60	7·84	7·10	3·83	3·90	22·38
Piché „	4·35	7·26	8·40	10·65	9·86	8·55	7·79	6·50	4·63	3·92	28·10

Table VI.

Time	Dry shaded soil*	Shaded air	Wet bulb	Black bulb *in vacuo*
6 a.m.	26·5	23·0	14·5	35·0
7	28·2	28·5	15·6	47·2
8	30·0	31·7	17·3	57·5
9	32·5	34·0	18·5	63·0
10	35·2	36·0	19·3	70·0
11	37·6	38·6	19·8	72·0
Noon	39·2	40·0	20·2	73·0
1 p.m.	39·8	41·0	20·6	74·0
2	40·2	41·5	20·8	73·5
3	40·5	41·3	20·1	69·0
4	40·5	41·0	20·3	62·2
5	39·0	39·0	20·3	48·5
6	37·5	37·0	20·4	34·7

* One inch below surface of powdered soil.

[1] Observations extending over so long a period as 90 minutes may fail to record the maximal severity of the conditions to which the plant is occasionally exposed. In the Gezira damage does not usually extend beyond a narrow strip along the north side of a block of cotton, and it may therefore be presumed that gusts of hot dry wind do not usually last very long. It will, however, be of interest to make a closer study of the resistance of cotton to these extreme conditions and of their frequency throughout the year.

to depression of a wet bulb thermometer, although this was situated in a meteorological screen distant some 250 yards from the scene of the experiment.

Table VI contains temperature observations made in the course of this experiment.

(B) *Relation of total losses from water and from soil to Piché loss.*

The total evaporation in 24 hours shown by a Piché tube suspended beside the aluminium boxes was 27·00 mm., whereas the reading of the Piché tube inside a meteorological screen was two-thirds of this amount. The larger figure corresponds to 107·0 imperial tons per acre and to 112·5 cubic metres per feddan. Losses from water and from moist soil were much smaller and are given below as percentages of this amount.

Table VII.

Free water surface		63·39 tons	59·2 % of Piché
Soil maintained at	30 %	32·47 „	30·4 „
„	25 „	27·74 „	25·9 „
„	20 „	22·36 „	20·9 „
„	15 „	17·12 „	16·0 „
„	10 „	11·11 „	10·4 „
„	5 „	1·49 „	1·4 „

It must be noted that the losses shown here for water and for moist soil do not correspond to losses in the field, owing to the comparatively high temperature of the dry soil in which the boxes lay (see Table VI).

IV. Relation between total water loss and moisture content for sand and for soil.

Boxes containing sand which had been moistened some days before were exposed beside those discussed in Sections II and III above. Moisture content of the sand and water loss (grams per 40 cm.² or imperial tons per acre) were related as shown in the following table which is comparable with Table III.

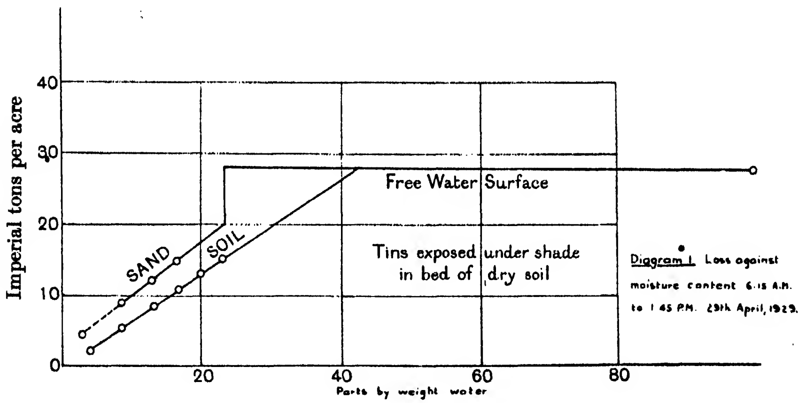
Table VIII.

Moisture content (%) ...	5	10	15	20
Period 1	0·53	0·97	1·24	1·31
2	0·71	1·50	1·87	2·02
3	0·79	1·69	2·25	2·85
4	0·97	1·91	2·78	3·60
5	0·95	2·10	3·37	4·53
Totals (periods 1–5) ...	3·95	8·17	11·51	14·31
Totals for soil (1–5) ...	0·81	5·24	7·94	10·16

At the foot of this table total losses for sand and for soil are given; the comparison unfortunately does not extend through the 24 hours, but no serious error is introduced by assuming that the ratio of loss in periods 1 to 5 to loss in 24 hours is the same for sand as for moist soil. On this basis we have the following figures:

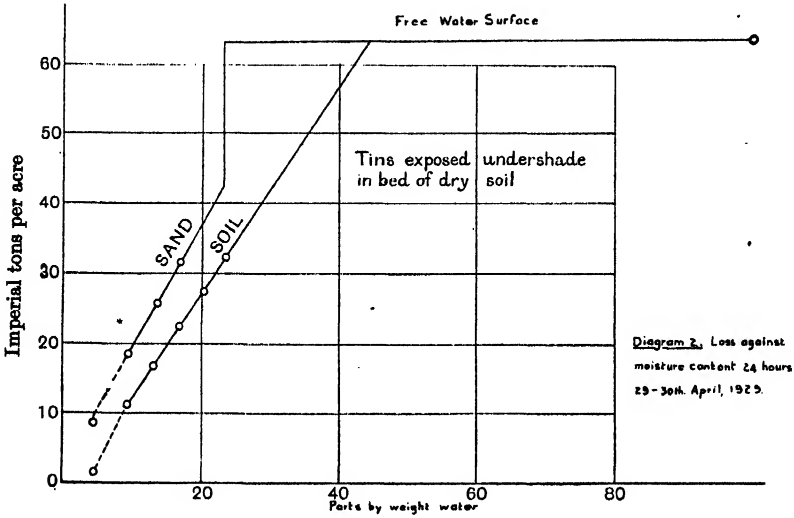
Moisture content (%) ...	5	10	15	20	25	30
24 hours' loss from sand ...	8·75	18·1	25·4	31·6	—	—
24 hours' loss from soil ...	1·49	11·11	17·12	22·36	27·74	32·47

These figures are plotted in Diagrams 1 and 2 against composition by weight of the mixture exposed. In the case of soil observed, losses other than the lowest are seen to lie on a straight line whose prolongation cuts the horizontal line representing loss from a free water surface



(28·01 and 63·39 tons per acre) at about the same place in the two diagrams. This indicates that Gezira soil does not behave like a free water surface until its moisture content reaches about 79 per cent. (on a dry basis). The observation is not at variance with the known water holding capacity of Gezira soil and an attempt will be made to obtain experimental confirmation. In the case of sand also the observed losses lie on a straight line whose prolongation cuts the level for a free water surface at about the same place in the two diagrams. The agreement shown here indicates that the calculation of 24 hours' losses for sand was not seriously at fault. The actual course of the curve must differ from that of soil since the pore space of the sand is completely filled when the proportion of water to sand is 23 to 77 by weight. This is reflected in the diagrams by a sharp break in the curves although some rounding off presumably occurs in practice.

The linear character of the experimental curves suggests the desirability of expressing moisture contents on a wet basis and subjecting all the observations for one period to a uniform analysis instead of grouping them as described in Section I. It should, however, be noted that in this series of observations transfer of heat from the aluminium boxes to the subsoil was largely eliminated, whereas in the field and in experiments incorporating field conditions such transfer of heat takes place and so reduces water loss during the day and causes the results to deviate from the linear relation now shown. The method of analysis will therefore be reconsidered when more data are available.



V. Comparison of loss in sun and in shade.

This comparison was made under more natural conditions in that tins containing moist soil or water were pressed flush with the surface of a ridge whose adjacent furrows had been heavily watered the day before. The experiment was made on February 18th, 1929, and the periods of exposure were as follows:

(*v*) from 7.30 a.m. to 9.15 a.m.
(*w*) from 9.30 a.m. to 11.15 a.m.
(*x*) from 11.30 a.m. to 1.15 p.m.
(*y*) from 1.30 p.m. to 3.15 p.m.
(*z*) from 3.30 p.m. to 5.15 p.m.

One lot of boxes was exposed to direct sunlight while another lot was

screened by wooden boards one foot above ground level. The results of this experiment are given in Table IX.

Table IX. *Losses (grams per 40 cm.² or imperial tons per acre) from soil and water.*

	Exposed in shade				Exposed in sun			
Moisture content (%)...	20	30	40	Water	20	30	40	Water
Period (v)	1·1	1·1	1·2	2·6	2·1	2·1	2·5	4·1
(w)	2·1	2·7	3·1	5·6	3·2	4·7	5·5	8·9
(x)	2·3	2·9	3·5	8·6	4·0	5·4	6·8	12·8
(y)	1·7	2·5	3·2	6·8	3·7	5·2	5·7	10·2
(z)	1·2	1·9	2·6	5·6	2·6	3·6	3·7	8·3

When these figures are expressed as percentages of the total loss in the five periods of exposure, it is again found that moist soil and water show similar responses to variations in atmospheric conditions. To obtain a general idea as to the effect of insolation on water loss, it is therefore permissible to take horizontal means from Table IX thus giving approximately equal weight to losses from soil and from water. These means are shown below:

Table X.

Period	Mean loss in shade	Mean loss in sun	Difference
(v)	1·50	2·70	1·20
(w)	3·37	5·57	2·20
(x)	4·32	7·24	2·92
(y)	3·55	6·20	2·65
(z)	2·82	4·54	1·72

These relations are illustrated in Diagram 3. It will be noticed that even during the day the effect of insolation as measured by the difference between loss in the sun and loss in the shade is less than the latter. If allowance is made for the considerable evaporation which occurs at night, it is found that the drying power of the air, as measured by shade losses, is about twice the drying power of direct sunlight as measured above. Some observations on this are made in Section VI; at present, it may be noted that the maximum evaporation attributed to insolation corresponds to absorption of energy at the rate of 2000 to 2500 kilo calories per square metre per day. We have at present no means of measuring the incidence of solar radiation, but the kindness of Prof. P. Vageler placed at our disposal some data from which it was found that the total incidence at ground level at Wad Medani on the day of this experiment was in the neighbourhood of 6000 kilo calories per square metre. It is hoped that further work along these lines will afford a clearer view of the way in which such an increment of energy is utilised.

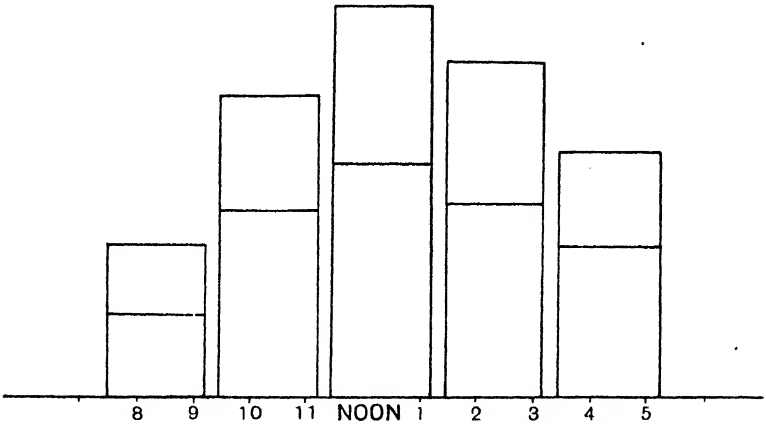


Diagram 3. Mean loss in sun (whole columns) compared with mean loss in shade (lower portions of columns).

VI. Water loss from fallow soil.

Reference to Table II shows that soil of low moisture content exposed in aluminium boxes and resting on a bed of dry soil picked up 0·4 gm. per 40 cm.^{2} in the nine hours between 9 p.m. and 6 a.m. In confirmation of this result it was found that samples scooped from the top inch of fallow soil varied in moisture content according to the time of day. Thus the mean moisture content of 96 samples collected at noon on twelve days was 3·76 per cent. whereas that of samples collected at 7 a.m. was 5·26 per cent. It appears therefore that there is a daily fluctuation in the moisture content of fallow soil.

This observation is of interest from more than one point of view. The great drying power of the air to which reference is made in the preceding section is evidently derived from its passage over vast stretches of parched sun-baked soil, but why that power should persist in the middle of an irrigated area some 500 square miles in extent was less obvious. The fact is, however, that for most of the cotton season at any rate the larger part of this area is dry and functions as a combined oven and desiccator. It is notoriously difficult to ascertain the surface temperature of soil, but it probably does not fall far short of black bulb temperatures which, as shown in Table VI, may exceed 70° C. The comparatively small difference in water loss shown by shaded and unshaded soil is therefore due to the fact that a large increment of energy is carried to both by the moving air.

It is also of interest to note that a part of the energy falling on fallow soil is stored as chemical energy and liberated at night as heat of hydration. A sample of Gezira soil was examined by the method of Bouyoucos (4) with the following results:

Moisture content of soil (%)	Gram calories per gram
0	8·75, 8·85, 9·85
1·26	5·51, 5·95
2·94	3·47
3·41	3·46
3·52	2·65
5·03	1·92
5·80	1·67
6·66	1·13
6·74	1·29
7·84	0·99
8·85	0·37
11·1	0·65
11·7	0·35

As moisture content increases the amount of heat liberated falls off exponentially and within the range 3·76 to 5·26 per cent. is about 1 gram calorie per gram. If therefore the top inch of soil weighs 100 tons per acre, the energy liberated is about 100 ton calories per acre which if wholly used in evaporation elsewhere is equivalent to about 0·2 tons. It is doubtful whether data of this kind can be put to quantitative use in studying evaporation, but it may at least be said that in the Gezira hydration contributes to the nightly flow of heat from soil to air.

The fact that the uptake of water in the field exceeds that occurring when dry soil is exposed at night in a box supports the view that the loss occurring in the day is partly made good by diffusion of water vapour from the lower layers with the result that slow progressive desiccation occurs. McKenzie Taylor and Burns (5) tentatively ascribe the beneficial effect of fallow in Egypt to modification of the colloidal properties of the soil. It is possible that the cumulative effect of these small daily changes in moisture content is similar to that discussed by Haines (6) when the variation in moisture content is much greater and much less frequent.

It may finally be noted that during the passage of air over a field of cotton its drying power must suffer a gradual reduction which is absent when small tins are exposed in the manner now described. This would limit the application of experimental data to field problems had it not been shown (Section III) that losses from tins are closely related to losses recorded by a Piché evaporimeter. Exposure of Piché tubes in a given area will accordingly provide a simple means of allowing for this gradient in atmospheric conditions.

Summary.

The severe climatic conditions under which cotton is grown in the Gezira make it desirable to establish relations between meteorological data and irrigation practice. By means of a rapid and fairly accurate balance it is practicable to measure water loss when small boxes containing moist soil are exposed for short periods and under different conditions. The scope of this method has been ascertained by preliminary experiments as to (1) the dependence of water loss on moisture content of the soil, (2) the distribution of water loss through the day, (3) the comparison of water loss from sand and from Gezira soil, and (4) the comparison of water loss from shaded and unshaded soil. The application of data so obtained to field problems is facilitated by the fact that in respect to distribution and amount losses from soil are related to loss shown by a Piché evaporimeter. Within the canalised area alternation of irrigated and fallow land is responsible for local variations in atmospheric conditions. A daily fluctuation in the moisture content of surface soil from fallow land is recorded and may be instrumental in favourably modifying its physical properties. The heat of wetting of Gezira soil has been measured.

Our thanks are due to Dr A. F. Joseph under whose direction this work was done and to Prof. P. Vageler who visited these laboratories and kindly placed at our disposal data which he had collected as to the apparent value of the solar constant at ground level.

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A NOTE ON THE DETERMINATION OF SOIL ORGANIC MATTER.

A WET COMBUSTION METHOD.

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Introduction.

In a recent article which appeared in this *Journal* (1), Robinson, McLean and Williams have described a simple method for determining organic matter in soils, which is, in effect, an adaptation of the Kjeldahl procedure. They have found (contrary to expectation), that the sulphur-dioxide evolved when various soils, sucrose and cellulose are heated with concentrated sulphuric acid represents the equivalent of the carbon oxidised, according to the equation:

$$C + 2H_2SO_4 = CO_2 + 2SO_2 + 2H_2O.$$

Hence, by estimating the liberated sulphur-dioxide iodometrically, the carbon content of the material under examination can be determined. Their results appear to justify their contention, and demonstrate that, under the conditions of the experiment, no appreciable decomposition of sulphuric acid occurs through direct heating. An advantage of the new method is that both carbon and nitrogen contents may be determined in a single Kjeldahl digestion. This should greatly facilitate the compilation of much-needed data for carbon/nitrogen ratios of soils, humus, peats, leaf-mould, organic manures and the like, and should lead to greater knowledge of the effect of environmental conditions on the decay of plant residues, and of other questions of agricultural importance.

The need of rapid quantitative methods of soil examination is being felt more and more as the need for fuller exploration of the soil environment in relation to growth and behaviour of crop plants becomes evident, and as more detailed soil surveys are being required. The routine examination of the large numbers of soil samples that must periodically be collected in connection with ecological studies and detailed field-to-field soil surveys, necessitates the introduction into soil analysis of rapid methods, not necessarily of the highest degree of accuracy, but nevertheless reliable, simple, and easily mastered, even by workers of little

previous training. The method for determining organic carbon devised by Robinson, McLean and Williams is therefore very welcome at this juncture; and should find wide application in soil studies.

A WET COMBUSTION METHOD.

During the past eight years, the writer has employed a wet combustion method for determining organic matter of soils in the West Indies. The procedure was first brought to his notice by Sir Francis Watts, then Imperial Commissioner for Agriculture in the British West Indies. It is described by Watts in his monograph on the soils of Dominica (2). The procedure is based on a method used by Cross and Bevan (3), which was adopted as apparently meeting the requirements of soil analysis. The original description by Watts is here presented:

The operation consists in treating the substance under investigation with concentrated sulphuric acid followed by chromic anhydride, when a mixture of carbon dioxide with some carbon monoxide is evolved. This is measured, and the amount of carbon in the gas ascertained by calculation. As pointed out by Cross and Bevan, there is an advantage in performing a gasometric analysis, in that similar volumes of carbon monoxide and dioxide contain equal weights of carbon.

The analysis is performed as follows: About 2 grams of *very finely powdered soil*, or as much as may be expected to yield about 90 to 100 c.c. of gas, are placed in a small flask of about 50 c.c. capacity, having a side-tube in the neck. To this, 10 c.c. of concentrated sulphuric acid are added, and mixed by gently shaking. The flask is now attached by means of the side-tube and a piece of stout rubber tubing to a suitable apparatus for measuring gas, described below. When first attached, the flask is brought into such a position that the neck is horizontal. A platinum boat containing chromic anhydride is placed in the neck, and the neck closed by means of a rubber stopper, care being taken that the neck is not soiled during the introduction of the chromic anhydride, so as to ensure that none comes into contact with the rubber stopper. While in this position, the apparatus is allowed to assume the temperature of the air. When this is accomplished, the gas measuring apparatus is adjusted.

The gas measuring apparatus consists of a nitrometer with a three-way cock connected with a reservoir of mercury and a manometer tube. To adjust the apparatus, the nitrometer is filled with mercury by raising the mercury reservoir while the nitrometer is in communication with the outside air through the appropriate channel of the stop-cock. The flask is now also brought into communication with the outside air by means of the stop-cock. Thus the contents of the entire apparatus are at atmospheric pressure. The flask is now brought into communication with the measuring apparatus by a proper turn of the stop-cock. The flask is then brought into a vertical position, whereby the chromic anhydride falls into the flask. It is brought into intimate contact with the soil and acid in the flask by gentle shaking, whereupon gas is at once given off. The flask is now immersed in a bath of boiling water to complete

the reaction, and is agitated occasionally. In about twenty minutes, the evolution of gas ceases, the hot water bath is removed, the apparatus allowed to resume the temperature of the air, the mercury levels in the measuring apparatus accurately adjusted, and the volume of gas evolved measured. This volume is simply that found in the measuring apparatus, and is independent of that contained in the flask and its connexions, which remains constant.

As there is always a slight evolution of carbon dioxide on the addition of the sulphuric acid, this to a certain extent saturates the liquid with gas. It has not been customary therefore to make any correction for the solubility of the gas in the acid used, as suggested by Cross and Bevan.

From the volume of the gas evolved, after correction for temperature and pressure, the weight of carbon is easily calculated.

This process is preferable to that in which the soil is digested with bichromate of potassium and sulphuric acid, and the evolved carbon dioxide absorbed by caustic soda and weighed. It has been shown by Cross and Bevan that, under these conditions, the carbon is underestimated, owing to the formation of carbon monoxide which escapes absorption. It is also much more convenient than determining the carbon by combustion in oxygen, for in this case, the discrimination between organic carbon and carbon of earthy carbonates is troublesome.

No attempt is made to estimate the humus as such, the organic carbon multiplied by the factor 1·724 being regarded as humus, or perhaps more properly, as potential humus. In the tropics, where decomposition of organic matter is rapid and continuous, there appears to be good reason for adopting this course, for vegetable matter newly added to the soil in the form of green dressing or of pen manure, usually takes little time to exert a beneficial action. The estimation of the total organic carbon therefore appears to be preferable to estimating the humus only.

The writer has little to add to this clear description of a wet combustion method, except (*a*) that a porcelain boat may be substituted for one of platinum; (*b*) that for soils containing appreciable carbonate, it is advisable to submit them to a preliminary treatment with dilute hydrochloric acid or with acetic acid; (*c*) that three sets of apparatus, allowing three separate determinations of carbon content to be made simultaneously, may be successfully employed; (*d*) that the method gives satisfactory results in the hands of untrained assistants who have had no previous knowledge of quantitative chemical analysis, and (*e*) that, with these modifications, a determination of the carbon content of a soil can be made in an average time of about fifteen minutes.

EXPERIMENTAL.

In order to test the wet combustion method recommended by Watts and to compare with the method suggested by Robinson, McLean and Williams, the following experiments were conducted.

(1) *Comparison of the wet combustion method with the method of Robinson, McLean and Williams for soils.*

Some experimental results obtained (for soils) are set out in Table I. The figures show that the Kjeldahl procedure yields somewhat higher values for carbon contents of soils than the wet combustion method, which gives values that, on the average, are about 84 per cent. of the former, and are always the lesser.

Table I. *Comparison of the Watts wet combustion method with the Kjeldahl method of Robinson, McLean and Williams with soils.*

	Percentage carbon				
Soil no.	(*A*) Wet combustion		(*B*) Kjeldahl		$A/B \times 100$
V. 9	0·86 0·92	0·890	0·96 —		92·7
V. 10	0·25 0·25	0·250	0·25 0·32	0·285	87·7
V. 11	1·13 1·15	1·140	1·41 —		80·8
V. 12	0·18 0·19	0·185	0·20 0·27	0·235	78·7
OCP. II	0·64 — —		0·77 0·65 0·62	0·680	94·1
OX. 1	0·71 0·83 0·85	0·800	1·08 1·08 —	1·080	74·1
NPX. 26	0·71 0·94 —	0·825	1·06 1·09 1·09	1·080	76·4
NPX. 42	1·30 1·36	1·330	1·66 1·62	1·640	81·1
OCP. III	0·62		0·69		89·8
			Average ...		83·9

"Blank" determinations were run for each method, using pure ignited fine quartz sand instead of soil in every case. For the wet combustion method, the "blank" was always zero; but for the Kjeldahl method, it was found to vary between 0·0011 and 0·0028 gm. of apparent carbon, the higher value being obtained when the heating was continued two hours longer than is generally necessary in order to effect complete decolorisation of soil. (Compare the mean "blank" determined by Robinson, McLean and Williams, namely, 0·00107 gm. carbon (range, 0·00090 to 0·00125).)

In all our experiments, sulphur-dioxide was absorbed in Reiset towers, as recommended by Robinson, McLean and Williams, but even then,

contrary to their experience, a correcting "blank" appeared to be required.

(2) *Comparison of the two methods for carbonaceous substances other than soils.*

Robinson and his collaborators submit some interesting results obtained by their procedure with sucrose, starch, cellulose, carbon and humic acid. Unfortunately, they do not specify the kinds of cellulose and of carbon they examined. We have determined the carbon contents of several materials other than soils, and have found that considerable variations are shown by the data. Some results are presented in Table II.

Table II. *Comparison of the two methods with carbonaceous materials other than soils.*

Material	Percentage carbon: Theoretical	(A) Wet combustion		(B) Kjeldahl	% recovery (A)	% recovery (B)
Sucrose	42·1	39·1, 39·4, 39·6	39·4	(39·6)*	93·6	94·1
Starch	44·4	41·8, 43·0, 43·0	42·6	(42·4)*	95·9	95·5
Celluloses:						
(*a*) Cotton wool	44·4	40·0, 43·8	41·9	(41·9)*	94·4	94·4
(*b*) Filter paper	44·4	39·9, 40·7, 43·7	41·4	(41·9)*	93·3	94·4
Carbons:						
(*a*) Wood charcoal† ...	95·7	88·4, 95·0	91·7	83·9	95·8	86·6
(*b*) Graphite†	35·3	45·1, 46·3	45·7	1·5 (4·9)	129·4	4·2 (13·9)
(*c*) Animal charcoal† ...	15·4	6·6, 7·3, 7·9	7·3	7·9	47·4	51·3
(*d*) Carbon from sucrose‡	100·0	18·1, 19·5, 20·1	19·2	60·3, 61·2 → 60·7	19·2	60·7

* Figures in brackets are Robinson, McLean and Williams' values.

† The *wood charcoal* used contained 4·32 per cent. ash. The *graphite* used contained 64·66 per cent. ash. The *animal charcoal* used contained 84·56 per cent. ash.

‡ The sucrose charcoal was obtained by treating sucrose with concentrated sulphuric acid followed by thorough washing.

It will be noted that the percentage recovery for the carbohydrates is almost as high with the wet combustion method as with the Kjeldahl

procedure. For *wood charcoal* (4·32 per cent. ash) it is higher with the wet combustion method. For *graphite* (64·66 per cent. ash) it is above 100 with the wet combustion method, but very low with the Kjeldahl method, the higher value (in brackets) being obtained in a six hours' digestion instead of three hours. We are unable to explain this difference. It cannot be due to mineral carbonate, for this would affect each result similarly. For *animal charcoal* (84·56 per cent. ash) the two methods yield similar results, but for *sucrose charcoal* the wet combustion method gives much the lower value.

Discussion.

The fact that the Kjeldahl procedure, as recommended by Robinson, McLean and Williams, yields, in general, somewhat higher results than the wet combustion method adopted by Watts when applied to *soils*, suggests that sulphuric acid at temperatures approaching the dissociating point of the compound (300° C.) is a slightly better oxidising agent than a mixture of sulphuric acid and chromic acid at 100° C. Indeed, according to Schollenberger (4), in order to effect complete oxidation of the carbonaceous substances present in soils by the use of an aqueous mixture of sulphuric, phosphoric, and chromic acids, a temperature coinciding with the boiling point of the mixture is required. A similar opinion is expressed by White and Holben (5), who employed aqueous sulphuric-chromic acid mixtures at boiling point. Under these conditions, carbon recoveries approximating 100 per cent. were obtained by these investigators, both for soils, manures, grain-meals, peat and charcoal.

Nevertheless, the experimental results yielded by the milder sulphuric-chromic acid treatment suggested by Watts appear to justify its employment in routine soil analysis. The procedure is somewhat simpler than the Kjeldahl method introduced by Robinson, McLean and Williams, it is more rapid, it requires no "blank" determinations, it needs less supervision, and it involves a fewer number of separate operations.

The chief advantage offered by the Kjeldahl method is that nitrogen determinations can thereby be made at the same time as the carbon estimations are being run.

Acknowledgment.

The writer wishes to acknowledge indebtedness to his colleague, Mr G. Rodriguez, who performed most of the analyses recorded in this paper.

SUMMARY.

(1) A gasometric method for the determination of organic carbon in soils by the use of sulphuric-chromic acid mixtures at 100° C., as suggested by Watts in 1902, is described, and recommended for use in routine soil analysis.

(2) The method is compared with the Kjeldahl procedure recently introduced by Robinson, McLean and Williams, and is found to give results which are generally similar, though numerically slightly lower.

(3) The wet combustion method is somewhat simpler than the Kjeldahl method, but it has the disadvantage that nitrogen determinations cannot be made simultaneously, so that its usefulness in studies on soil organic matter is decidedly less.

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SOME ASPECTS AND METHODS OF SOIL SURVEY WORK.

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THE particular method adopted for surveying the soil of a region naturally depends upon the objects of the survey. These may be classified broadly as follows:

(1) To gain general knowledge of the distribution of the main soil-types over a relatively unknown territory; preliminary reconnaissance survey.

(2) To gain more exact knowledge of the soil-types, for the purposes of selecting sites for agricultural experiment stations; of aiding future land development, and of delineating areas suited to particular cropping systems; broad ecological survey.

(3) To gain detailed knowledge of the soil conditions that govern plant-growth, in particular, the growth of any specific crop-plant; physiological-ecological survey.

(4) To gain information which will be of use in solving problems in geographical economics, hygiene and sanitation, road building, town planning and water-works construction; special-purposes survey.

(1) PRELIMINARY RECONNAISSANCE SOIL SURVEY.

Preliminary soil exploration is usually conducted simultaneously with or following previous topographical and geological surveys; it is greatly facilitated by access to reliable topographical and geological maps, if such are available, and to books of travel and commerce. Its rate of performance is largely decided by the existence of roads and rivers, and by the type of natural vegetation that covers the ground. The kind of geological map of most useful application to the reconnaissance survey is the drift map, especially for glaciated or alluviated regions. Where mature soil-types exist, as in tropical continents, detailed study of climatology and meteorology, based on records of rainfall, temperature changes, humidity, prevalence of winds, and sunshine, should systematically be undertaken.

The reconnaissance surveyor should avail himself of the experience of native tribes and of early settlers regarding the fertility of the soils when planted with various crops or exploited by shifting cultivation.

The preliminary reconnaissance soil maps should delineate sedentary and transported soils, and should indicate to what extent geological composition has impressed itself upon the soil-types, or how far climatic factors have obliterated the specific effects of geological influences.

(2) BROAD ECOLOGICAL SOIL SURVEY.

It is now widely recognised that reliable information as to the distribution of soil-types, and of the main complexes of soil factors that control plant growth, can be gained by regional studies of natural vegetation. The methods that have been or might be employed in such studies are described in a recent book published by the British Empire Vegetation Committee, under the editorship of Prof. A. G. Tansley and Dr T. F. Chipp (26). The earlier works of Warming (28), Schimper (24) and Clements (5) afford abundant inspiration to the student of geographical ecology, and furnish an excellent theoretical basis for detailed field investigations.

During the mapping of the main plant communities, field studies of soil profiles should be made, and samples taken for subsequent laboratory examination. A field equipment, containing such reagents as hydrochloric acid, Comber's thiocyanate solution, and a simple colorimetric indicator-set, such as "Soiltex," or the B.D.H. outfit, will greatly aid in characterising soil-types in natural and artificial sections as well as in surface exposures. The broad ecological maps, drawn up during this work, furnish a useful guide in the formulation of plans for future agricultural development. As an example, the maps and descriptions of the soils and vegetation of Africa presented in Shantz and Marbut's book (25) might be cited, although the scope of this work is perhaps too broad to be of immediate service in the opening-up of undeveloped regions in Africa.

(3) DETAILED ECOLOGICAL SOIL SURVEY.

(1) Introduction.

Perhaps no phase of the soil survey has provoked more controversial discussion than the particular questions of the compilation of data and the elaboration of soil maps suited to the practical needs of the plant-grower.

The now familiar survey methods evolved and applied in 1896, by the United States Bureau of Soils, under the guidance of its first Director, Dr Milton Whitney, whilst fulfilling an important object, clearly cannot yield all the necessary soil data required by the farmer or planter (7). These methods were apparently based on the early conception that the

chief soil factor controlling plant growth is physical texture. Since soil texture is largely (though not wholly) decided by the actual dimensions and the proportionate amounts of the mineral particles that comprise a soil, it became customary to analyse by mechanical grading a large number of representative soil-samples collected over any area under survey. For each soil, some six to nine arbitrary fractions were thus differentiated, and the soil's physical composition was assessed by reference to a table of their weight percentages. Conventional names based on such tables of results were finally given to the soils, *e.g.* clay, silty-clay, clay-loam, sandy-loam, sand. The ultimate soil maps constructed from the analytical data generally showed the distribution of these main physical soil-types, but they failed to distinguish soils in good and in bad tilth.

It has frequently been maintained that such maps are of little value to the farmer or planter, in that they tell him no more than he can himself discover by "walking the land," and by observing the behaviour of soils during the operation of ploughing and cultivation. Furthermore, they are not sufficiently detailed to be of service in ecological work, since they usually depend on the examination of a relatively small number of composite soil-samples taken from rather widely-separated localities. Finally, they are somewhat costly to produce, and the accumulation of the necessary data is laborious and time-absorbing. Nevertheless, it must be conceded that the uniformity of procedure and nomenclature adopted by soil surveyors ensures that differences in personal judgment and opinion do not obscure the variations in soil-type that occur over areas too large to come within the experience of an individual observer.

Concurrent with mechanical soil analyses, it has long been customary to submit representative soil-samples to conventional chemical examination by methods largely based on the early conceptions of Liebig and of Dyer. That the results of such examination do not entirely depict the specific nutrient relations that exist between soil and plant, has been amply demonstrated by critical experiments conducted in the years 1904 to 1922, at Berkeley, California (16). The results of these experiments proved that there was no consistent relationship between crop-yield and (1) the *total quantity* of any element present in the soil; (2) the amount of any soil element *soluble in hydrochloric acid*; and (3) the amount *soluble in citric acid.* On the other hand, definite positive relationship between crop-yield and (4) amounts of soil elements (especially nitrate) *soluble in water* was demonstrated by a series of fortnightly determinations conducted over several growing seasons.

An important conclusion from the Californian experiments is that studies of the nutrient status of soil from the point of view of physiological ecology must be dynamic in aspect, that is, they must be systematically carried out over time-periods covering at least an entire growing season for any particular crop under investigation, or, better, over several seasons during which climatic factors may vary.

This exacting qualification enormously increases the work of the soil surveyor, and it is doubtful whether it could successfully be met in attempts to procure data that may be plotted on maps for the information of the practical plant grower. The detailed procedure might, however, be more generally undertaken in experimental plot-work at agricultural stations.

Fortunately, certain rapid methods have recently been evolved for examining soils, both in field and laboratory, and these deserve wider application than they seem so far to have received. The more serviceable of them are briefly discussed below, under the headings of the particular soil factors that they are intended to assess.

(2) Ecological soil factors.

For purposes of presentation, the chief soil factors that govern plant growth may be classified as follows (23):

(A) *Static factors* (not liable to fluctuation during a growing season).

1. Mechanical soil composition.
2. Soil-moisture constants.
3. Mineralogical soil composition.
4. Chemical constants, including lime-deficiency.
5. Organic matter content.
6. Mineral carbonate content.
7. Mineral phosphate content.
8. Certain harmful factors.
9. Penetrable depth for roots.

(B) *Dynamic factors* (fluctuating during a growing season).

1. Soil tilth; state of aggregation.
2. Gross soil-moisture content.
3. Water-supplying power of soil.
4. Nutrient-supplying power of soil.
5. Oxygen-supplying power of soil.
6. Soil temperature.
7. Micro-organic activities.

(3) Notes on the significance and measurement of some soil factors.

(A) *Static factors.*

1. By *mechanical soil composition* is implied (*a*) the average dimensions of the particles that comprise the mineral portion of the soil, and (*b*) the percentage amount of the chief grades of these particles. It expresses the degree of sandiness or of clayiness. It does not express the *arrangement* of the soil particles into flocks, aggregates or compound particles, nor the spatial relationship between colloidal and non-colloidal components.

Mechanical composition may perhaps be gauged sufficiently accurately for most purposes of ecological survey work by means of some single-value constant, such as the index of soil texture recently proposed by the writer (12). This index is derived from determinations of moisture content at the point of stickiness and of coarse and fine sand content. Data are easily obtained without the employment of elaborate apparatus by workers possessing no previous scientific training.

2. *Soil-moisture constants* include those that may have some bearing on plant growth (*e.g.* gravitational, capillary, and hygroscopic water-contents; wilting coefficient), and those that have no such direct relationship (*e.g.* moisture equivalent; maximum water-retaining capacity; moisture at the point of stickiness; air-dry moisture; hygroscopic coefficient).

Their determination gives some measure of certain static properties of soils by reference to their water-relations. Among those moisture constants that may be easily determined by unskilled workers are the maximum water-retaining capacity, by Hilgard's method (10), and the physical constants yielded by the Keen-Raczkowski procedure (17). These particular constants appear to be especially valuable for purposes of rapid soil characterisation.

3. *Mineralogical composition* of the particle-grades, as ascertained by petrographical methods, yields evidence of geological origin and of nutrient resources of soils (15).

4. *Chemical constants* include such data as ultimate, proximate, and partial chemical composition; silica-alumina ratio; combined water; exchange capacity; degree of unsaturation with reference to exchangeable ions; gross content of specific exchangeable ions, such as calcium and potassium; lime-deficiency or requirement; and degree of acidity or of

alkalinity. Standard methods for their determination are available, and need not be described here.

Lime-requirements may be determined very rapidly by the Hardy-Lewis method (14).

5. *Organic matter content* includes the numerous more or less arbitrary fractions that may be separated by laboratory processes, and which present varying degrees of resistance to decomposition and oxidation. It includes the *humus* fraction.

Total organic matter contents may be determined by the dry combustion method, which measures organic carbon. The experimental procedure, however, is tedious and lengthy. A modified Kjeldahl method for measuring carbon contents has been recently elaborated by Robinson, McLean and Williams (21). By its employment, both carbon and nitrogen contents of soils may quickly be determined. The method should prove of considerable value in soil survey work. Certain wet combustion methods (especially that described elsewhere by the writer (13)) are quite rapid and reliable, and appear also to be suited to the needs of the surveyor. Determinations of organic fractions, such as material soluble in caustic alkali (for example, Eden's modification of Grandeau's method (6)), have certain application to special problems. Picttre's pyridine-extraction method (20), according to the writer's experience, is unreliable, mainly because the presence of certain ions, such as calcium, appears to affect the dissolution of the pyridine-extractable components of soil organic matter.

6, 7. *Mineral carbonate and phosphate contents* provide sources of calcium, magnesium, iron, carbonate, and phosphate by which the soil may be replenished with these important components. Their measurement by conventional methods usually offers no special difficulties.

8. Among the *harmful factors* are usually considered: (*a*) excessive soil acidity and alkalinity, (*b*) abnormal concentrations of certain compounds or their ions (*e.g.* aluminium, iron (especially ferrous-iron), magnesium, manganese, zinc, lead, copper, borate), (*c*) organic toxins, and (*d*) undesirable micro-organisms (23). Few agricultural soils present these factors in harmful amount, so that they need not be considered here.

9. *Root penetrability* is largely decided by sub-soil conditions, and these should be studied if a complete knowledge of the environment be the aim of the ecological investigator.

The study of root distribution in the field offers many difficulties. The root excavation methods employed by Weaver in the United States (29), whilst they yielded interesting ecological evidence in the hands

of the originator, are solely qualitative in aspect, and therefore do not lend themselves to soil survey work. A quantitative method, which so far has given satisfaction, is being applied by the writer in a study of root distribution in sugar-cane soils. It consists in obtaining soil-cores containing roots by driving a sharp-edged steel tube into the ground at different distances from the plant. Core-samples to successive depths are procured at regular distances along a radius for each of several growing plants. The roots are easily separated from the soil-cores by soaking them overnight in water and afterwards washing them on a sieve. The root material is dried and weighed. It may be subdivided if desired into old, thick roots, and young, thin roots, each fraction being weighed separately. For the sugar-cane, soil-cores obtained at six successive six-inch depths at three points along a $2\frac{1}{2}$ ft. radius parallel to the surface drains, the boring being repeated for each of five plants (90 cores in all), seem to be sufficient to describe its root distribution. The soil material collected at the same time may be suitably mixed so as to form representative depth-samples for subsequent laboratory examination.

(B) *Dynamic factors.*

1. *Soil tilth* is an expression of the manner in which the composite discrete particles and colloidal material are associated and arranged in a soil. Its importance in soil relations can hardly be over-stressed, for it controls many important growth factors, such as penetrability by roots and water, and oxygen-supplying power. Its measurement in the field, however, is a matter of considerable difficulty. Promising results have been obtained by the writer by the use of a soil-compaction instrument which, in effect, functions like a Vicat needle. The number of strokes of an iron plunger-hammer, which operates within a tube, three feet in length, to drive a pointed steel "torpedo" into the soil, is recorded for various sites in different representative areas. At any one site, 32 determinations are made within a four-foot square, both for the top-soil surface, and for that of the sub-soil at the six-inch depth, exposed by removing the surface six-inch layer. At the time of measurement, representative soil-samples are taken for determinations of moisture contents. The experiment is repeated at each site several times during one or more growing seasons. The data obtained furnish evidence of change of tilth produced by wetting and drying, by tillage and by beating rain, as well as by other natural processes.

2. *Gross soil-moisture contents* convey information regarding the total

amount of available water and the degree of drainage of a soil at different periods. Their exact bearing on problems of plant-growth can only be appreciated when a soil's static moisture constants are known, and when its water-supplying power has been studied. They therefore furnish data that are of some comparative value, provided care be taken to obtain representative composite soil-samples, and to protect them from water-loss prior to weighing.

3. *Water-supplying power* of soil, a conception due to Livingston, has been discussed by the writer elsewhere (11). Briefly, it appears to be decided by the total available water (magnitude of water-reservoir of gross moisture content), and by the conductance of a soil for water. It is clearly dependent on soil texture and tilth. It may be measured directly by the soil-point method, in which an "artificial root," presenting a uniform water-absorbing surface, is placed in contact with the soil and allowed to take up water for a definite period of time. Results are most easily obtained with Livingston porous-porcelain cones; writing pencils appear to exhibit too much individual variation as regards the water-absorbing property. In an experiment, five or ten porcelain cones are inserted within a four-foot square marked out on a selected site. The experiment is repeated within each site at different times in a growing season. The results obtained possess especial significance during the onset of drought conditions. Some typical data are presented in Table I, for

Table I. *Fluctuations in water-supplying power of soils of good and of bad plots.*

Date	Water-supplying power (mg. per 10 sq. cm. per hour)		Difference	Significant difference	Gross soil-moisture content (% oven-dry soil)	
	Good plot	Bad plot			Good plot	Bad plot
1928, April 27	210	14	196	102	18·0	21·8
May 8	248	22	226	158	18·8	22·6
June 22	1920	729	1191	462	20·0	24·6
June 23	1109	221	898	408	20·2	24·9
June 28	915	284	631	437	21·2	26·5
Aug. 4	1272	982	290	95	25·6	33·2
			At wilting point		9·6	15·1
			At saturation		28·2	43·4

Note. During the height of the *dry season* in April, the "bad plot" soil almost reached permanent wilting level, whilst the "good plot" still held a large reserve of available soil moisture. The rainy season began in early June.

two sugar-cane plots, one producing good crops and the other poor crops (8). The plots are situated within 300 yards of each other in the same cane-field. Evidently, water-supplying power furnishes a single-

value constant which may be used to compare the water status of soils, particularly during drought.

4. *Nutrient-supplying power* (*i.e.* the ability of a soil to deliver nutrients to roots at a rate requisite for the maintenance of a suitable concentration of ions or molecules at the absorbing surfaces) is a conception similar to the last. Its determination by a similar method, however, is liable to considerable inaccuracies. Nevertheless, some measure of the rate of nutrient supply may be obtained by laboratory examination of periodic soil-samples by the electrical-conductivity method of Atkins (2). Some typical data are presented in Table II (9). They refer to the same sugar-cane plots as those mentioned in reference to water-supplying power. The conductivity figures are decided by the resultant effect of several electrolytes dissolved out of the soil. In order to assess the relative concentration of each, detailed chemical analysis would have to be made. This is not usually necessary, however, in ecological survey work, where the ease and rapidity of experimental methods are the main requisite. Water-soluble phosphate can be easily determined by the Atkins coeruleo-molybdate method (1).

Table II. *Fluctuations in nutrient supply of soils of good and of bad plots.*

	Readily available nutrient supply			Rate of supply of potential nutrients		
Date	Good plot	Bad plot	Difference	Good plot	Bad plot	Difference
1926, Nov. 2	109	76	33	62	27	35
Dec. 7	125	66	59	—	—	—
Dec. 14	113	73	40	91	24	67
1927, Jan. 4	110	55	55	125	14	111
Jan. 11	128	59	69	—	—	—
Jan. 25	130	62	78	—	—	—
Feb. 8	102	56	46	137	14	123
Feb. 23	143	73	70	115	1	114
Mar. 11	87	62	25	86	31	55
Apr. 7	89	68	21	43	9	34
Apr. 19	117	51	66	64	12	52
May 3	121	72	49	131	14	117

The figures are specific electrical conductivity values $\times 10^6$.

Note. Greatest differences in rate of supply were shown during the wet weather that prevailed in January and February, 1927. The differences were markedly reduced during a dry spell in March and April.

In order to obtain more detailed information of the nutrient status of soils than is yielded by electrical conductivity measurements, *electro-dialysis* may perhaps be applied. By employing several cell-units, this process might be adapted to the simultaneous examination of a large number of soil-samples, and might thus serve as a routine method in physiological ecology. In this way, fluctuations in exchangeable base

contents might be followed throughout a growing season for purposes of soil comparison. Recent investigations (19) have demonstrated that such fluctuations for certain adsorbed ions (such as those of calcium and of potassium) are to be expected.

5. *Oxygen-supplying power* appears to be closely linked with water-supplying power and with soil temperature. Investigations of the oxygen relations of the root system of the cotton plant, conducted by Cannon in California (3), have demonstrated that the oxygen supply of moist soil operates along with soil temperature in controlling root growth. Thus, if the supply of oxygen greatly exceeds the absorption capacity of water for oxygen at a given temperature, so that the physiological requirements of the root system are fully met, an increase in oxygen partial pressure of the soil atmosphere might not be followed by a corresponding increased rate of growth (4).

In soils lacking adequate aeration, there probably exists definite equilibrium relationships between partial pressure of oxygen in the soil atmosphere, concentration of dissolved oxygen in the soil water, and oxidation-reduction potential in the environment of the plant root. Hence, studies of oxygen supply in soil should involve considerations of oxidation-reduction conditions, which, so far, appear not to be amenable to simple direct measurement.

There has so far been no suitable method evolved for measuring the oxygen-supplying power of soils in the field. The pyrogallol-absorption method, described by Livingston and Hutchins (18), appears to be too complicated for this purpose. The ecological investigator is thus obliged to have recourse to merely qualitative procedures or to measurements of water-table fluctuations in the sub-soil.

Among the qualitative methods, soil-profile studies appear to offer possibilities. Thus, in many Trinidad sugar-cane fields, sub-soil water fluctuations appear to have produced a characteristic fine-mottling or speckling in the "B" horizon. The speckled layer occurs at about 26 inches below the surface in a "good" cane plot, and at 10 inches in a "bad" plot. Water-table levels may be followed throughout the year by sinking perforated, gauze-covered iron pipes into the soil at various sites in a field or plot, and making monthly or weekly measurements of the depth of the water-surface in them. From the figures obtained, maps showing soil-water isobaths may be constructed for the different seasons of a year. Such maps should serve to guide the farmer or planter in the laying-down and testing of drainage systems.

6. *Soil temperature* is liable to fluctuation according to topographic

aspect, presence or absence of vegetative cover, colour of soil, depth of soil stratum, texture, water-content, and rate of evaporation at the surface (23). It usually shows a marked lag when compared with corresponding air temperature, and is not so prone to extreme variation. Its effect on plant growth is difficult to assess, since so many environmental factors are affected by alterations in temperature.

7. *Micro-organic activities*, which depend on the magnitude and composition of the soil fauna and flora, as well as on soil conditions, may apparently vary between very wide limits, even within the period of a single day (22). Their effects on the nutrient-supply of soil may be assessed by periodic determinations of specific soil components, particularly ammonia, nitrite and nitrate, as well as of certain of the more available substances comprising soil organic matter. Hence, as far as ecological soil studies are concerned, it is generally not necessary to know the biological composition of the soil fauna and flora, nor to be acquainted with the fluctuations in the numbers of its individuals.

(4) Application of experimental results to the construction of soil maps.

From the foregoing considerations of the experimental methods of physiological ecology, it appears that certain soil constants, that assess the factors controlling plant growth, may quickly and easily be determined. It remains now to suggest how the results obtained may be applied in the construction of soil maps.

Of the *static soil factors*, physical character and reaction (degree of acidity or of alkalinity) may be regarded as fundamental and of primary importance. Both may readily be measured by routine methods; the one by determining the "index of texture"; the other by means of the quinhydrone electrode. Results may be obtained sufficiently quickly by these methods to allow of the examination of a very large number of soil-samples in a relatively short time. Hence, by collecting and examining closely-spaced "spot" samples, the surface soil of a large area may be clearly characterised, and even the minor variations detected. The experimental data may be plotted in arbitrary schemes of colours, such as those already suggested by the writer (12).

Other static soil factors, such as certain moisture constants, lime-requirement, organic matter content, and carbonate and phosphate contents, may perhaps also be measured sufficiently quickly and easily to allow of their routine determination on individual spot-samples, for plotting on soil maps.

On the other hand, it may not be feasible to undertake a large amount of laboratory work, such as is necessary for the detailed determination of the static soil factors belonging to this second group, and to the remaining groups above-mentioned. If this be the case, then recourse may be made to a scheme of *composite grouping*, whereby the large number of spot-samples are reduced to a few representative samples suited to the particular laboratory facilities that are available for their examination. The writer has elaborated and used such a scheme of grouping; its brief description here may be of service to others. The procedure is as follows:

A method of spot-sample grouping.

The spot-samples are first broadly classified according to their "indices of texture" (I.T.) into (1) sands (I.T., 0–24); (2) loams (I.T., 25–39); (3) silts (I.T., 40–49); (4) clays (I.T., 50–60).

(The number of these groups might, of course, be multiplied, if thought desirable.)

Secondly, the spot-samples *within each textural group* are classified according to their reaction values (pH).

(In practice, it has been found more satisfactory and theoretically sounder to employ *exchange* pH values, rather than *normal* pH values, for classifying on the reaction basis. Exchange pH values are determined in suspensions of the spot soil-samples in molar potassium chloride solution by the quinhydrone electrode method.)

Convenient exchange reaction groups are: (1) intensely acidic (*exch.* pH, 3·0–3·9); (2) highly acidic (*exch.* pH, 4·0–4·9); (3) markedly acidic (*exch.* pH, 5·0–5·9); (4) slightly acidic (*exch.* pH, 6·0–6·9); (5) alkaline (*exch.* pH, 7·0–7·9).

(It will be noted that "reaction indices" may readily be derived from exchange pH values by merely subtracting two units (2) from the initial pH figure.)

A typical example of the application of the suggested scheme is given in Table III, which indicates how the spot-samples obtained are grouped according to the above rules. The estate lies on estuarine alluvium[1]. It comprises some 880 acres of arable land. One spot-sample was taken in about every 8½ acres, which is less than the area of an average cane-field.

[1] In cases where various, easily-recognisable geological formations are represented within an area, the surface soil of the different outcrops might be sampled separately.

Table III. *Example of spot-sample grouping for purpose of constructing representative composite soil-samples.*

Woodford Lodge Sugar Estate, Trinidad. (Canelands Survey, 1928.)

(1) Sands Exchange reaction groups					(2) Loams Exchange reaction groups					(3) Silts Exchange reaction groups					(4) Clays Exchange reaction groups				
1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
.	9	47	59	46	52	10	18	51	44	.	8	7	48	.	.	74	.	.	.
.	45	53	65	246	.	55	43	.	247	.	11	49	240	.	.	101	.	.	.
.	70	54	82	.	.	60	57	.	.	.	50	62	243	.	.	102	.	.	.
.	71	69	249	.	.	66	58	.	.	.	56	63	.	.	.	103	.	.	.
.	81	72	.	.	.	67	61	.	.	.	64	78	.	.	.	105	.	.	.
.	253	73	.	.	.	68	83	.	.	.	75	80	.	.	.	.	.	.	.
.	254	84	.	.	.	85	87	.	.	.	76	94	.	.	.	.	.	.	.
.	255	248	.	.	.	86	90	.	.	.	77	97	.	.	.	.	.	.	.
.	.	250	.	.	.	88	110	.	.	.	79	244	.	.	.	.	.	.	.
.	.	256	.	.	.	113	111	.	.	.	89	.	.	.	.	.	.	.	.
.	.	.	.	.	.	114	115	.	.	.	91	.	.	.	.	.	.	.	.
.	.	.	.	.	.	238	116	.	.	.	92	.	.	.	.	.	.	.	.
.	.	.	.	.	.	251	239	.	.	.	93	.	.	.	.	.	.	.	.
.	.	.	.	.	.	252	242	.	.	.	95	.	.	.	.	.	.	.	.
.	.	.	.	.	.	257	245	.	.	.	96	.	.	.	.	.	.	.	.
.	.	.	.	.	.	258	.	.	.	.	98	.	.	.	.	.	.	.	.
.	.	.	.	.	.	260	.	.	.	.	99	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	100	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	104	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	106	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	107	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	108	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	109	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	112	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	237	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	241	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	259	.	.	.	.	.	.	.	.
.	8	10	4	2	1	17	15	1	2	.	27	9	3	.	.	5	.	.	.
	WCP I	WCP II				WCP III	WCP IV				WCP V	WCP VI				WCP VII			

Notes on Table III.

The total number of spot soil-samples obtained within the area is 104. Of these, 24 belong to the sand category, 36 to the loam, 39 to the silt, and 5 to the clay category. Of the *sands*, none are intensely acidic; 8 are highly acidic; 10, markedly acidic; 4, slightly acidic; and 2 are alkaline. Of the *loams*, 1 is intensely acidic; 1, slightly acidic; 2, alkaline; and the rest (17 and 15), highly or markedly acidic. The *silts* include no intensely acidic, and no alkaline soils. Of the *clays*, all are highly acidic. Thus 87 per cent. of the soils of this estate are highly or markedly acidic.

Composite soil-samples, for determinations of chemical constants (organic matter, lime-requirement, phosphate, exchangeable bases, total

nitrogen, etc.), are constructed by mixing equal parts of each of the spot-samples belonging to the several different groups (columns). Thus, samples 9, 45, 70, 71, 81, 253, 254, 255 (highly acidic sands) comprise composite sample WCP I (types (1), (2), *i.e.* "index of texture" between 0 and 24, and exchange reaction between pH 4·0 and pH 4·9).

Where the number of representatives of any one texture-reaction group is small, it may not be worth while to make up a composite sample for that group. Thus, in the table, groups containing less than five spot-samples are ignored. The total number of composite samples for the sugar-estate under consideration is seven, and these are taken as representative of the main soil-types within that area.

Objection may be raised against the assumption that spot-samples are best grouped for purposes of detailed laboratory examination into composites according to their indices of texture and their reaction values. The following arguments are therefore presented in favour of this procedure:

(i) Both mechanical composition and reaction of soil appear to be decided by geological origin and by the extent to which pedogenic processes have operated. Thus, when weathering and leaching have been profound, the surface soil of the mature profile that develops is typically sandy and highly acidic, both the finer mechanical fractions and the soluble bases having been removed, no matter what the original rock material may have been. On the other hand, the mechanical composition and the reaction of a relatively little weathered soil varies with the nature of the parent rock. In either case, the two attributes, composition and reaction, show close relationship with soil-forming processes.

(ii) Mechanical composition and soil reaction (particularly exchange reaction) mainly decide the magnitude of static soil factors, such as lime-deficiency or requirement, degree of base unsaturation and exchange capacity.

(iii) Extensive leaching may bring about marked reduction in carbonate and phosphate contents, whose residual magnitudes may thus be a function of degree of acidity.

(iv) Organic matter contents appear also to be partly decided by pedogenic processes, which, moreover, largely account for the type of organic matter produced (27). This seems to be the case particularly in certain virgin lands recently brought into cultivation (grass-lands, park-lands, maritime and swamp-lands), and in arable lands that have received little addition of plant residues, or have been dressed uniformly and regularly over a period of many years.

It may be admissible therefore to employ composite samples, constructed in the manner described above, instead of individual spot-samples for determination of many of the static soil factors, and to use the data obtained for plotting on field maps that show the locations of the spot-samples. Thus, a value say for lime-requirement, obtained by examination of any one composite sample, may be regarded as a mean of the lime-requirement values of all the spot-samples contained in that composite sample, and no appreciable error may be introduced in taking this mean value as the lime-requirement figure for each of the spot-samples for purposes of plotting.

The data may be entered on field plans as coloured splotches. The maps may be left as spot-maps, or adjacent splotches of the same colour may be joined together. Such completely coloured maps may finally be copied in water-paint washes, with suitable legends attached to them[1].

In constructing ecological soil maps, distinction should clearly be drawn between static soil factors and dynamic factors. For an area undergoing detailed ecological survey, several different sorts of coloured maps might be prepared, for example:

(A) *Static soil factor maps* ("Permanent" maps).

These require no further description. They might severally show the distribution of the following factors:

1. Soil composition (index of texture).
2. Soil reaction (normal or exchange reaction).
3. Lime-deficiency or lime-requirement.
4. Total organic matter content.
5. Maximum water-retaining capacity.

(B) *Dynamic soil factor maps* ("Variable" maps).

Before preparing maps showing fluctuations in the several dynamic soil factors that control plant growth, decisions must be reached as to how many factors are to be considered, and how frequently these factors are to be assessed and recorded. The simplest procedure is to select

[1] Instructive soil models may be made *directly* from the spot soil-samples, if these have been collected at sufficiently close intervals (*e.g.* one or more for every field of a farm or estate), by mixing a little of the soil material with some sort of colourless varnish (cellulose acetate dissolved in amyl acetate, or "Necol" label varnish, give excellent results). The mixture is painted on to a plan of the area over the field or site whence the sample was taken. The finished "patch-work" model shows soil-colour variations, as well as extremes of mechanical composition.

representative sites or plots within the area under investigation, and to concentrate entirely on these sites throughout one or more growing seasons. The sites may best be selected by observations on the growing crop, by measurements of crop-yields, or by botanical analysis of the plant population. Among the dynamic factors that may then be measured at each site, the following appear to lend themselves to periodic mapping:

1. Gross soil-moisture content.
2. Water-supplying power.
3. Nutrient-supplying power.
4. Nitrate content.
5. Water-soluble phosphate content.
6. Soil temperature.
7. Water-table level.
8. Surface and sub-surface soil tilth.

Ready means of plotting the data on to field plans will suggest themselves to the investigator. The various possible procedures need not be further discussed here. Although the work involved may be regarded as laborious and difficult, it is the writer's experience that, with proper organisation, the results are easily accumulated, and certainly do furnish reliable indications of deficiencies, both manurial and cultural, for any particular areas that may be regarded by the practical plant-grower as requiring amelioration. The agricultural and economic ends appear to justify the ecological means, and the soil investigator is enabled thereby to co-operate more closely with the farmer or planter in his efforts to increase the productivity of his lands.

SUMMARY.

(1) Soil surveys are classified into (i) preliminary reconnaissance surveys, (ii) broad ecological surveys, (iii) detailed physiological-ecological surveys, and (iv) special-purposes surveys. The objects of each are briefly discussed.

(2) Particular consideration is given to the methods of physiological ecology, which attempt to assess the chief soil factors controlling plant growth.

(3) Soil factors are grouped into (A) static factors, which do not fluctuate appreciably during a growing season, and (B) dynamic factors that may exhibit marked fluctuations within a season, or during years when climatic conditions vary.

(4) The significance of, and some experimental methods for measuring, certain of these growth factors, both in the field and in the laboratory, are briefly noted.

(5) The application of experimental data to the construction of soil maps is discussed. It is suggested that maps displaying static factors (such as mechanical composition, soil reaction and lime status) should be clearly differentiated from periodic seasonal maps that depict fluctuations in certain dynamic factors (such as water and nutrient-supplying power), amenable to direct simple measurement.

Both sorts of maps should have practical value in that they convey exact information regarding the more permanent soil characters and the manurial and cultural requirements of areas believed to require amelioration.

(6) The employment of spot-samples and of composite samples constructed therefrom in soil examination is described, and a tested method of sample grouping is presented and illustrated.

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PROTEIN AND MINERAL METABOLISM IN PREGNANT SOWS ON A NORMAL OR HIGH CALCIUM DIET COMPARED WITH A CALCIUM-DEFICIENT DIET.

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(With One Text-figure.)

Introduction.

A study of the metabolism and utilisation of ash ingredients by pigs is of special importance in view of the fact that the rations of swine are composed mainly of cereal grains which are known to be deficient in lime. Nutritional literature shows that not only does a deficiency in any particular mineral element cause retardation of growth and prevent optimum production, but that under protracted deficiency, pathological symptoms develop and disease appears.

Researches on the protein and mineral metabolism of swine have been confined to the young growing animal, and nothing is known about the requirements for minerals during pregnancy. In the past it has been generally assumed that the foetal requirements—both for energy and minerals—are suitably met, provided the organic constituents of the ration are made adequate. The tendency under modern conditions of intensive production to confine brood sows to paved or concrete yards, with limited access to soil or green food, makes it imperative that more definite information be available on the special drain imposed on the maternal tissues by the developing foetus.

The effect of pregnancy on protein and mineral metabolism.

The effect of pregnancy on metabolism has been studied mainly from the point of view of human nutrition. The experimental animals have in most cases been dogs and rabbits though a limited number of investigations have been carried out on human pregnancies. With one or two exceptions no work is recorded on farm animals. A summary of the earlier work is given in Marshall's *Physiology of Reproduction*, 1922. The work on the nitrogen balance during pregnancy is very conflicting. Hagemann (1) carried out observations on pregnant bitches and showed that

during the first month of gestation there was a negative nitrogen balance, but that during the second half of pregnancy nitrogen retention occurred. He concluded that the new organism was formed at the expense of the maternal tissues. Ver Eeke (2) carried out similar work with rabbits and his results often indicated these two phases. Even on the same diet, however, he sometimes obtained a positive and sometimes a negative balance of nitrogen at different stages of gestation. That this variability may be due to the level of protein intake is shown by the experiments of Jägerroos (3). In Ver Eeke's experiments the nitrogen was only slightly above maintenance requirements. Jägerroos (3) showed that in the dog on a high protein intake (pure flesh) there was a positive nitrogen balance each week except the second. On a lower nitrogen intake (flesh) a negative nitrogen balance occurred only in the fifth and sixth weeks. On an intake of protein just sufficient for equilibrium, after the first few days, a loss of nitrogen occurred each week throughout gestation, except in the third week.

An important contribution to the subject was made by Bar and Daunay (4) who, instead of supplying pure flesh, incorporated in the diet bread, fat, beef, salt and water. They also analysed the young and afterbirths and so calculated the nitrogen storage of the foetus. They found that the period of gestation was triphasic. At first there was a period of nitrogen retention, followed by nitrogen equilibrium or a slight negative balance, but towards the end of gestation retention increased each week. This occurred in the three bitches worked with, as was observed to be the case in two of the animals in Jägerroos' experiments. Of great importance is their conclusion that "pregnancy in a healthy animal on a rational and sufficient diet does not necessitate a drain on the maternal stock of nitrogen to satisfy the needs of the foetus." This conclusion was later supported by the work of Murlin (5) and both observers agree that the greatest nitrogen retention occurs in the second half of pregnancy when the demand of the foetus is greatest.

In 1900 Michel (6) published the analysis of human embryos and foetuses at different stages and of a new-born infant. Up to the seventh month there was a gradual increase in the deposits of nitrogen, ash, phosphate and lime, followed by a sudden increase late in foetal life. Calculating from the analysis of Michel, Hoffstrom (7), from his studies of the metabolism of a human female, showed that while 101 gm. of nitrogen had been utilised by the foetus, the maternal organism had gained to the extent of 209 gm. of nitrogen during pregnancy. Again, in 1912 Landsberg (8), on the same basis, found in human pregnancy a

retention of nitrogen four times as great as that required for the foetus. Further confirmation is given by the work of Wilson(9), who showed that nitrogen retention occurred in excess of the requirements of the foetus, mammary glands and genitalia of the mother.

Crowther and Woodman(10) have also studied the nitrogen balance of a cow during pregnancy. They found that on a ration containing protein in excess of the ante-pregnant requirement there was a loss of nitrogen in the early stages of pregnancy and that the rate of storage was not very considerable until within three or four weeks of parturition. They consider that "the demands made on the food protein for the single purpose of foetal development were relatively small, but from their data on storage and excretion of nitrogen (including calf and placenta) they show that a deficit of 277 gm. of nitrogen must have been supplied from the maternal protein. The fact, therefore, that the cow had to make use of organised tissue products for the nutrition of the developing foetus suggests that although the protein content of the diet was in excess of the ante-pregnant requirement, yet it was not sufficient for the pregnant animal. Crowther and Woodman suggest that more generous feeding than was given in their investigation is obviously required in actual farm practice.

Harding(11), in his review of metabolism in pregnancy, suggests that loss of nitrogen in the initial stages of pregnancy is characteristic of the lower animals and that nitrogen conservation only occurs in the human female.

The effect of pregnancy on mineral metabolism.

As early as 1899 Hugouneng studied the retention of ash ingredients by the human foetus and showed that during the last three months of gestation the foetus acquires twice as much mineral matter as previously. He showed that the fixation of sodium decreased while that of calcium, phosphorus and potassium increased; the former by the replacement of cartilage by bone and the potassium with the increased manufacture of red-blood corpuscles. Ver Eeke, from his work with rabbits mentioned above, states that in the 16 cases studied, a very marked decrease in phosphate elimination occurred during the last week of pregnancy. Further, in twelve out of the sixteen cases, he found the retention of phosphate higher in the first and last weeks than at any other time during gestation, the last week's retention being higher than the first. Jägerroos had also noted a rough parallelism between nitrogen and phosphate retention, increased retention taking place in the second half of pregnancy.

Hoffstrom, however, observed that during pregnancy the organism showed a greater tendency to retain nitrogen than phosphorus and that there was no strict parallelism. He found that a storage of 56 gm. of phosphorus occurred in human pregnancy of which 18 gm. were deflected to the foetus and adnexa while 38 gm. were retained by the mother organism. He also states that the requirement for lime is particularly high during the last three months, but that pregnancy does not necessarily entail a loss of calcium by the mother. Hoffstrom computes that 30·1 gm. of calcium was utilised by the foetus and that there was a net gain of 4·2 gm. calcium by the mother.

The work of Dibbelt (12) on pregnant bitches is also interesting in that he found that, on a diet poor in calcium, the foetus does not suffer a loss of calcium, the deficiency being made up entirely by the maternal bones and tissues. Schkarim (13) carried out similar work with bitches and he also found that the effects of the maternal diet (both vegetable and animal) on the composition of the young were not pronounced.

The influence of high lime intake on skeletal formation in the foetus of swine has been studied by Hart and his associates (14). Their records indicate a very uniform relation between dry skeleton produced and weight at birth, but no evidence is given that high calcium intake by the mother influenced in any degree the weight of the skeleton. Further, from their analysis of the bones, the percentage proportion of calcium deposited by the mother before birth in the skeleton of the foetus was in no degree influenced by the supply furnished in the ration. They also failed to find any relation between the intake of lime and the amount of calcium in the soft tissues of the foetus.

The work done on mineral metabolism, therefore, indicates that it is possible for the maternal organism to retain ash ingredients in excess of the foetal requirement and so set up a reserve supply for parturition and lactation.

OUTLINE OF EXPERIMENT.

The present work may be regarded as an extension of the experiments started by Mr H. R. Davidson, M.A., at the Cambridge University Farm, Gravel Hill, in 1924. The original object of the investigation was to enquire into the conditions that determine fecundity in swine, and one of the factors studied was the influence on fecundity of the lime content of the ration. For this purpose, a number of gilts of the "Large White" breed were bought in 1924 and divided into two groups, one group being placed immediately on a low calcium diet while the other

was given exactly the same dietary ingredients but with the addition of ground limestone. Both groups were treated in an identical manner, being confined to concrete pens and having no access to soil or green food, but with plenty of straw for bedding. These gilts were bred and allowed to farrow, several consecutive litters being obtained from each sow. The sows had therefore been on their respective rations for a considerable time before the third and fourth litters were produced; an important factor in the case of the calcium-deficient sows since any reserves of lime stored in the skeleton prior to the beginning of the experiment would have been considerably depleted by the first litters and subsequent lactation.

The gilt piglings of the offspring—forming therefore the second generation—were, after weaning at seven to eight weeks old, placed on their experimental ration. These were again bred and produced the subsequent pens which formed the third generation. The animals used for the work described in this paper were from the second and third generations only and were typical of the group they represent.

The basis of rationing.

The method of rationing adopted by Mr Davidson is based on the figures given in Prof. T. B. Wood's book on *Animal Nutrition*. The rations were designed according to the requirements of pigs growing at the rate of one pound live weight increase per day. These requirements are given in Table I.

Table I.

Live weight (lb.)	Digestible crude protein (lb.)	Starch equivalent (lb.)
56	0·34	2·05
100	0·51	2·89
150	0·60	3·37
200	0·63	3·73
250	0·67	4·19
300	0·70	4·25
350 (and upwards)	0·71	5·30

The food ingredients used were barley meal, maize meal, bean meal and blood meal. In rationing each of the two groups, the protein requirements were as nearly as possible met for different live weights, while the starch equivalent was only slightly above the requirement.

In addition to this, in order to correct the sodium deficiency of cereal grains and eliminate the possibility of any result obtained being due to this factor, both groups were given approximately one pound of common salt for every 200 lb. of mixed meal.

The data available on the lime requirements of pregnant sows is very scanty, and for feeding the normal or high-calcium group in this experiment the basis adopted was to supply 0·02 lb. lime (CaO) per pound of live weight increase[1], which is the figure given by Orr (15) in his paper on "The Importance of Mineral Matter in Nutrition."

The details of rationing were computed as follows:

Rations for normal or high-calcium group.

Ingredients	Parts	Digestible crude protein	Starch equivalent	CaO	P_2O_5
Barley meal ...	94	6·10	66·60	0·055	0·733
Maize meal ...	94	6·66	76·60	0·011	0·596
Bean meal ...	8	1·60	5·26	0·015	0·091
Blood meal ...	14	10·18	8·81	0·028	0·049
Ground limestone (85 % $CaCO_3$)	3·5	—	—	1·666	—
Salt (NaCl) ...	1·0	—	—	—	—
Total ...	214·5	24·54	157·27	1·775	1·469
Per cent. ...	100	11·43	73·30	0·827	0·684

Rations for calcium-deficient group.

Ingredients	Parts	Digestible crude protein	Starch equivalent	CaO	P_2O_5
Barley meal ...	94	6·10	66·60	0·055	0·733
Maize meal ...	94	6·66	76·60	0·011	0·596
Bean meal ...	8	1·60	5·26	0·015	0·091
Blood meal ...	14	10·18	8·81	0·028	0·049
Salt	1·0	—	—	—	—
Total ...	211·0	24·54	157·27	0·109	1·469
Per cent. ...	100	11·63	74·54	0·052	0·696

It will be observed that the ration of the calcium-deficient group, although seriously lacking in lime content, is in every other respect satisfactory. It is also noteworthy that approximately 88 per cent. of the ration consists of barley meal and maize meal in equal proportions, these being the common feeding-stuffs used for pig feeding in this country. Further, the proteins are supplied from four different sources and include both animal and vegetable proteins so that both the quality and quantity supplied are satisfactory.

It is possible that the ration might be criticised from the point of view of its vitamin content. To meet this objection it was decided to give the experimental animals cod-liver oil to supply vitamins A and D and also orange pulp as a source of the antiscorbutic vitamin C. The cod-liver oil incidentally supplied a certain amount of iodine, while the feeding of blood meal, which is rich in iron, prevented a deficiency in this important mineral element.

[1] According to Orr, the body of a store pig contains 1 per cent. of CaO. Therefore 1 lb. contains 0·01 lb. CaO and assuming 50 per cent. is retained, the food should contain 0·02 lb. CaO per lb. of live weight increase, or in our ration, per day.

The pregnant animals worked with in this investigation were given 7 lb. of mixed meal per day together with one-quarter of an ounce of cod-liver oil and 150 c.c. of orange pulp. They were fed twice daily, the food being allowed to soak for a few hours prior to feeding. The animals when not in the metabolic crate were given tap water, but while the balances were being taken, distilled water was supplied three times daily.

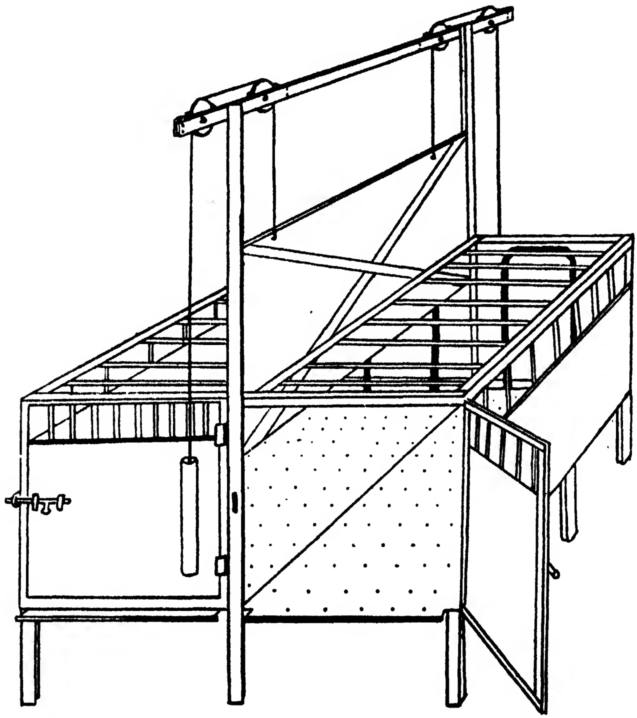

Fig 1.

DESCRIPTION OF METABOLIC CRATE.

The metabolic crate used for determining the mineral balances of these pregnant sows was specially designed by Dr H. E. Woodman. The chief feature aimed at was to give the animals complete freedom of movement—an essential factor in dealing with pregnant animals—while at the same time secure as complete a separation of urine and faeces as was possible. The crate had also to be constructed so as to be strong enough to stand a sow weighing from 500 to 600 pounds.

The material used for making the crate was galvanised iron. The general arrangement and construction may be gathered from Fig. 1.

The crate is divided into two halves by the door, which slides upwards and downwards by means of weights and a pulley arrangement, so that the animal could be moved from one portion to the other during the cleaning up operation. Each half is entered by means of a separate door which is bolted and also barred at the top and bottom. The floor of the crate is perforated with holes half a centimetre in diameter. This ensured a rapid and complete drainage of urine. Underneath the floor is a sliding hopper which can be removed and cleaned out at the beginning and end of the period. The urine drained from the hopper into vessels placed to receive it. The faeces were dropped on the floor of the crate and collected every half hour; a procedure which enabled an almost complete collection of faeces to be obtained without admixture with urine or hair.

The troughs used for feeding purposes were placed outside each section and were protected by lift-up doors so that the animal could not urinate into its trough. The latter were screwed on to wooden supports and were arranged so as to be level with the floor of the crate. The supports were secured to the crate by means of two bolts.

During the first period of the experiment it was found advisable to give the animal the full freedom of the whole crate, though a full grown sow could turn round in each section. After being in the crate a couple of days, the sows then acquired the habit of lying down in one section and urinating and defacating in the other. The result was that the animals came out of the crate quite clean and no scrubbing or washing was required. The sliding partition was found to be of great help since it enabled the crate to be cleaned out without undue disturbance of the sows.

In the literature dealing with the construction of crates for metabolic work with swine, one often comes across the statement that pigs if given complete freedom of movement will devour their own faeces. During the course of this work, seven different sows were put in the crate and out of these only one touched its faeces. This was repeated in the case of this particular sow when she was put in the second time and consequently she had to be discarded.

The chief objection to an iron crate for metabolic work with swine is that—since no straw could be given for bedding—the crate is rather cold, especially in winter. It was therefore found necessary to warm up the room and keep the temperature continually at about 60° F.

Experimental procedure.

With one or two exceptions, the animals were kept in the crate for a period of ten days. The first three days were regarded as preliminary during which the animals became accustomed to their surroundings and no collection of excreta was made. The actual seven-day experimental period—when composite samples of all excreta were kept and analysed,—was divided into two sub-periods of three and four days respectively. Composite samples of faeces, urine and washings for each sub-period were kept and analysed separately. The average figures obtained were then taken to represent the balance between the intake and output of food ingredients. It is important to note that the sows had been on their respective rations "from the time they were weaned at seven weeks old."

At the commencement and end of each sub-period, the floor of the crate and the hopper were rinsed out clean with distilled water using a long rubber squeegee. The floor of the crate was also rinsed out daily. Aliquot portions of faeces, urine and washings were taken daily into cold storage. By keeping the washings apart from the urine, a clear sample of the latter was obtained for analysis.

The life-histories of the animals worked with[1].

The normal or high-calcium group.

1. *Normal Sow* 8 N 2. The dam of this sow was put under experiment on October 27th, 1924, 8 N 2 coming from her first litter. She therefore belongs to the second generation and was farrowed on June 26th, 1925. She was first bred on March 5th having previously shown signs of oestrus on February 12th when 31 weeks old. She farrowed on June 27th, having 13 pigs of average live weight 2·13 lb. The sow had a good supply of milk, but when the piglings were weaned at seven weeks old, their average live weight was only 14·75 lb.

She was bred the second time on August 25th, 1926, and farrowed on December 18th. She had six pigs, four alive and two dead, the average live weight at birth being 2·12 lb. All the pigs, however, died two days after birth, though the sow had plenty of milk and farrowed quite normally. Her third litter was farrowed on May 10th and consisted of 8 pigs of average live weight 2·4 lb. When weaned at seven weeks old, the six pigs left had an average weight of 30·1 lb., indicating excellent development.

[1] These are abstracted from the records kept by Mr H. R. Davidson.

The sow, when bred the fourth time on July 7th, was put in the metabolic crate on July 11th, August 19th and October 11th. She farrowed on October 29th having four pigs, all alive, of average weight 2·95 lb. When weaned on December 24th the average weight of the pigs was 22·10 lb.

After weaning, this sow did not show signs of oestrus and when slaughtered on March 11th she was found to have cystic ovaries, very enlarged, with no ovarian tissue on one side and only three small corpora lutea on the other side. The small size of her fourth litter is possibly associated with this factor.

2. *Normal Sow* 7 S 1. This sow is from the same dam as 8 N 2 but belongs to the third litter of the second generation. Her first onset of oestrus occurred on December 28th, 1926 (32 weeks old), but she was not bred until March 27th, 1927. When she farrowed on July 16th she had ten pigs alive and one dead. The average live weight of the litter at birth was 2·84 lb. and at weaning 22·91 lb.

She was again served on September 19th, 1927, and was put in the metabolic crate on October 19th, November 21st and December 16th. She farrowed on January 10th, 1928, having 15 pigs, all alive and of average weight 2·7 lb. The pigs, however, could not be made to suckle though the sow had plenty of milk and was quite well and active herself. Six of the pigs were dead in six hours after birth, some of them having been presumably lain on. Six more pigs died by January 11th while the remaining three died soon afterwards.

The sow was again served on January 22nd and placed in the crate on January 26th. She turned, however, in three weeks' time and was not served again until March 5th. She was again put in the crate on March 9th.

3. *Normal Sow* 10 S 2. This sow belongs to the third generation and was farrowed on June 16th, 1926. She was first bred on March 29th, 1927 (38 weeks old). Her first litter was born on July 22nd and consisted of four pigs and one atrophic foetus (0·1 lb.). The average weight of the litter was 2·82 lb. and at weaning 22·35 lb.

After being bred again on November 24th, she was put in the crate on January 16th and February 28th. When she farrowed on March 18th she had ten pigs, all alive, of average weight 2·6 lb. The sow had a good udder and the young piglings thrived well.

From these notes it will be observed that the ration of the normal pigs was quite satisfactory for reproduction and lactation, and no harmful effect could be traced to the fact that the sows had been periodically

in the metabolic crate. It will, therefore, be of interest to compare the life-histories of these sows with those on the lime-deficient ration.

The calcium-deficient group.

4. *Sow* 77 N 8. This sow is from the second litter of one of the original group put under experiment and was farrowed down on July 21st, 1925. She showed obvious signs of malnutrition soon after birth as manifested by a dry and wrinkled skin and periodical stiffness of the hind quarters. The first signs of oestrus occurred on April 27th when she was 41 weeks old. Her first litter was farrowed on August 14th (107th day), ten pigs being born of which seven were dead and three alive. The average weight of the latter was 1·73 lb. but they all died the next day. It is significant that no milk could be seen before or during farrowing though a little could be squeezed out the following day. The udder was quite soft and flabby, and was in striking contrast to those of the normal gilts. Farrowing was very prolonged and the sow could not stand up for two or three days afterwards.

She was bred again on October 14th and farrowed on February 3rd, 1927. Observations kept during the period of gestation indicate that she was "weak on her legs," and often refused her food especially towards parturition. Her litter, however, consisted of 13 pigs, all alive, and one atrophic. The average live weight of the pigs was also 2·77 lb., but in two days' time they were all dead, the sow showing no signs of milk before or after farrowing.

After she was bred the third time, February 15th, she was put in the metabolic crate on March 29th and May 2nd. She farrowed down on June 8th, 1927, having nine pigs of which six were born alive and three dead. It is surprising to find that the average live weight of the litter was 3·02 lb. She showed typical signs of calcium deficiency, however, in that her udder was flabby when she farrowed and the sow was very weak on her legs. Four of the pigs survived at weaning and had an average weight of 18·78 lb.

This sow was served again on August 23rd, 1927, and put in the crate on November 11th and December 1st. She again went off her food at intervals before she farrowed on December 15th. Fifteen pigs were farrowed of which twelve were born alive, this again indicating that the periods spent in the crate had no harmful effect. The average live weight of the litter was 2·89 lb., but all the pigs were dead in four days' time, presumably from lack of milk by the mother although it was noted that she had a better udder than was usual for these calcium-deficient

sows. No milk, however, could be seen, and, owing to the difficulty in farrowing, the sow could not get up for four days and refused food.

The sow was bred the fifth time on February 13th and was put in the crate on February 17th. She was slaughtered on March 19th when it was found that she had 17 normal foetuses with three atrophic.

5. *Calcium-Deficient Sow* 32 p 3. This sow was from the same dam as 77 N 8 but comes from the third litter of the second generation which was farrowed on January 3rd, 1926. This litter was weaned on February 23rd and the records indicate that on the 27th, 32 p 3 was showing signs of stiffness. By June 22nd she showed what appeared to be obvious symptoms of rickets. Her first observed oestrus was on September 10th (33 weeks) and she was then getting stiffer in her joints with her legs bending inwards, these being symptoms typified by this group of pigs.

Her first litter was born on December 28th, 1926, nine pigs being born, four of which were atrophic and all dead. The sow had great difficulty in farrowing and had no milk. She was served the second time on February 14th and put in the metabolic crate on March 11th, April 22nd and May 13th. When she farrowed on June 4th, she had eight pigs of average live weight 2·90 lb. Notes taken on July 11th indicate that "the litter was losing condition fast, the piglings were dull and restless with dirty, scurvy skins." Six pigs were alive when weaned at seven weeks old and the striking fact is brought out that their average live weight was then only 9·62 lb. Immediately after weaning, the sow was returned to the crate.

The sow was again bred on August 23rd but only one balance could be taken during this gestation period. She farrowed twelve pigs on December 14th, ten of which were alive and of average live weight 2·57 lb. All the pigs, however, died the next day and the sow could not stand and feed normally until December 22nd. She was served again on January 3rd and a period was taken on February 6th. When slaughtered on March 12th she had 14 normal foetuses.

6. *Gilt* 64 S 7. This gilt was from the third litter of one of the original sows put under experiment, being born on July 1st, 1926. She was put in the crate on May 24th and showed sign of oestrus on the 26th (44 weeks old). During the last two days of the period she started devouring her faeces. She was bred on June 22nd and again put in the crate, but started eating her faeces the second time so she had to be discarded.

7. *Gilt* 58 S 7. This gilt was a litter sister of 64 S 7. She was typical of the calcium-deficient group in that her skin was rough and scaly

and she was often "off her feet." She was put in the crate on June 6th, but when taken back on the 17th she started fighting with another gilt and was badly injured. She did not show signs of oestrus until October 16th, 1927 (66 weeks), and when she was served, her legs gave way and she only got back into her box with difficulty. When slaughtered, it was found that she had a broken vertebra.

From these records it will be observed that the main manifestations of calcium deficiency—as typified by all the pigs in the group—were: (1) Rough scaly skins, dirty in appearance. (2) The pigs were periodically "off their feet" with their legs bending inwards, and developed what appeared to be characteristic signs of rickets. (3) The sows often refused their food towards parturition this indicating nutritive disturbances. (4) They had great difficulty in farrowing and could not stand on their feet for two or three days afterwards. (5) No signs of milk could be seen before or after farrowing, and their udder was very flabby especially in comparison with the normal sows. (6) Very few pigs survived at weaning time and even the few left made very poor live weight gains. It should be observed, however, that the sows themselves when they farrowed appeared to be in good condition.

It will be interesting here to compare the live weight of the piglings, of both groups, at birth and weaning time. These are given in Table II.

Table II.

No. of sow ...	8 N 2	7 S 1	10 S 2	77 N 8	32 p 3
1st litter ...	*(13) 2·13	(10) 2·84	(4) 2·82	(3) 1·73	(0) —
2nd litter ...	(4) 2·12	(15) 2·70	(10) 2·60	(13) 2·77	(8) 2·90
3rd litter ...	(8) 2·40	—	—	(6) 3·02	(10) 2·57
4th litter ...	(4) 2·95	—	—	(12) 2·89	(0) —

Average for 68 normal pigs = 2·55 lb. Average for 52 Ca-def. pigs = 2·74 lb.

Weight in lb. at weaning time (7 weeks old).

No. of sow ...	8 N 2	7 S 1	10 S 2	77 N 8	32 p 3
1st litter ...	(9) 14·75	(7) 22·91	(4) 22·35	(0) —	(0) —
2nd litter ...	(0) —	(0) —	—	(0) —	(0) —
3rd litter ...	(6) 30·10	—	—	(4) 18·78	(6) 9·62
4th litter ...	(4) 22·10	—	—	(0) —	(0) —

Average for 30 pigs = 22·44 lb. Average for 10 pigs = 14·15 lb.

* Figures in brackets denote size of litter (born alive).

It is surprising to find that at birth the calcium-deficient piglings were as heavy as those belonging to the normal group of sows. This supports the observation of other investigators "that if the diet is deficient in any ingredient, it is the maternal organism that suffers and not the developing foetus." The live weights at weaning time, however,

clearly demonstrate that the lack of milk on the part of the lime-deficient sows is reflected either by the fact that no pigs have survived or by a very poor development compared with those of the normal group.

The adequacy of the ration for reproduction and the good condition of the sows at parturition may be gathered from the live weight data given below.

Normal Sow 8 N 2	Weight in lb.	Normal Sow 7 S 1	Weight in lb.	Calcium-deficient Sow 32 p 3	Weight in lb.
July 11th ...	415	Oct. 19th ...	443	March 11th ...	331
July 26th ...	436	Oct. 31st ...	449	March 21st ...	350
Aug. 19th ...	469	Nov. 21st ...	498	April 22nd ...	398
Aug. 29th ...	475	Dec. 1st ...	508	May 2nd ...	401
Oct. 5th ...	527	Dec. 16th ...	529	May 13th ...	416
—		Dec. 20th ...	537	May 24th ...	439
Increase in weight in 84 days = 112 lb.		Increase in weight in 62 days = 94 lb.		Increase in weight in 74 days = 108 lb.	
Average increase per day = 1·33 lb.		Average increase per day = 1·51 lb.		Average increase per day = 1·46 lb.	
Calc. increase in weight in 115 days = 153 lb.		Calc. increase in weight in 115 days = 173·6 lb.		Calc. increase in weight in 115 days = 168 lb.	
Weight of litter at birth, 11·8 lb.		Weight of litter at birth, 40·5 lb.		Weight of litter at birth, 30 lb.	
Increase in wt. minus wt. of litter, 141·2 lb.		Increase in wt. minus wt. of litter, 133·1 lb.		Increase in wt. minus wt. of litter, 138 lb.	

Although the data given are too scanty to enable any conclusions to be drawn, yet the figures indicate that live weight is no criterion of the damage suffered during pregnancy on a calcium-deficient diet which is in every other respect satisfactory. Unfortunately, owing to their weak condition, it was impossible to weigh the calcium-deficient sows after parturition and therefore determine whether there was a loss of body weight as a result of gestation. The above data for 32 p 3, however, show that her increase in weight minus the weight of her litter compared favourably with the results for the two normal sows. This is in accordance with the fact that apart from stiffness, etc., the calcium-deficient sows were in good condition up to parturition.

THE CHEMICAL COMPOSITION OF THE FEEDING-STUFFS USED.

The chemical composition of the several samples of barley meal, maize meal, bean meal and blood meal are given in the appendix (Tables I–IV). It will be observed that considerable variation occurs in the composition of different samples of the same feeding-stuffs; particularly in barley meal, but for the purpose of this discussion it will

be sufficient to take the average figures only into consideration. These are given in Table III.

Table III. *Showing the average chemical composition of the feeding-stuffs (dry matter).*

Constituent	Barley meal	Maize meal	Bean meal	Blood meal
Crude protein	12·25	10·90	27·30	94·82
Ether extract	2·50	5·97	1·80	0·33
N-free extractives	76·72	79·08	59·96	1·89
Crude fibre	5·65	2·20	7·51	—
Ash	2·87	1·84	3·44	2·94
Lime (CaO)	0·1033	0·0190	0·2122	0·063
Phosphoric acid (P_2O_5) ...	0·9857	0·9516	1·023	0·252
Potash (K_2O)	0·6655	0·5165	1·489	0·362
Soda (Na_2O)	0·0332	0·0318	0·0430	1·128

The figures indicate the deficiency in protein and ash of barley meal and maize meal, the former being also low in oil content. Bean meal, on the other hand, is distinctly richer in protein and higher in ash. The main point of interest, however, is the unbalanced nature of the ash ingredients as shown by the excess of phosphoric acid over lime and of potash over soda. Of the three feeding-stuffs, bean meal, being a leguminous grain, contains twice as much lime as barley meal, but approximately ten times as much as maize meal which emphasizes the extreme deficiency of the latter in this important mineral element. Even in bean meal, however, the ratio CaO to P_2O_5 is only 0·20 while in sow's milk this ratio is 1·14 and in the skeleton of swine 1·30. Again, if the composition of the whole carcass be taken as a criterion, the lime is slightly in excess of the phosphate. Barley meal and maize meal are even more unbalanced than bean meal and the statement often made that "cereal grains do not allow the proper development of skeleton in pigs" can be readily understood from the above discussion.

The analysis of blood meal shows that this slaughter house by-product is exceedingly rich in protein but poor in ash and all mineral ingredients with the exception of soda. The feeding of blood meal, therefore, enabled the protein deficiency of the ration to be balanced with but slight disturbance in the amount and ratios of the ash ingredients. The type of blood meal used was specially chosen because of its low lime content, the commercial brands generally having a small proportion of bone meal incorporated in them.

Composite samples of orange pulp were also kept in cold storage and analysed so as to find to what extent the composition of the ration was

affected by the daily addition of 150 c.c. to the food. The results are given below.

Analysis of orange pulp.

Composite sample ...	I	II
Amount taken in c.c. ...	700	970
Weight in gm.	703	976
Moisture %	93·49	91·90
Composition of the dry matter per cent.		
Crude protein	20·94	13·22
Ether extract	3·22	3·88
N-free extractives ...	56·31	62·73
Crude fibre	10·50	11·54
Ash	9·03	8·63
Lime (CaO)	1·326	1·094
Phosphoric acid (P_2O_5) ...	0·8519	0·5973
Potash (K_2O)	3·443	3·027
Soda (Na_2O)	—	0·1887

The dry matter of the orange pulp is fairly rich in protein and ash, the latter, as would be expected, being deficient in sodium. The main characteristic of the ash is its high potash and lime content. No correction has been made in the balances for the ash ingredients supplied in the pulp since it varied considerably in juiciness with different consignments of oranges, and, moreover, a considerable quantity had to be evaporated to dryness so as to get a sufficient sample of dry matter for analysis. By calculating the amount on the basis of 92 per cent. moisture, however, it will be seen that the maximum daily increase in the lime and potash intake through the feeding of orange pulp was 0·16 gm. CaO and 0·41 gm. K_2O.

The protein and mineral balances of the two groups of sows.

The figures given in this paper represent the average results for the two sub-periods. For the purpose of discussion, the balance results for each individual element will first be considered, followed by a section on the degree of inter-relationship that was found to exist in the metabolism of the several elements.

The average daily nitrogen balances.

The average daily nitrogen balances together with the percentage of the intake excreted and retained are given in Table IV. It will be observed that in all the periods taken, the nitrogen retention is positive for both groups of sows, though considerable variation exists in the average daily retention of each individual. This indicates that the protein supply of the ration was both adequate and suitable for growth and reproduction.

Table IV. *Showing the daily nitrogen balances and the percentage of intake excreted and retained.*

Experimental no. of sow	Day of gestation	Intake of N in gm.	Gm. of N excreted in faeces	Gm. of N excreted in urine	Gm. of N excreted in washings	Total gm. N excreted	Total gm. N retained	% of intake in faeces	% of intake in urine	% of intake in washings	% of intake retained	Live weight increase per day (lb.)
Normal sow 8 N 2	13th	71·54	15·21	38·40	1·88	55·49	16·05	21·26	53·68	2·63	22·43	1·4
	49th	74·05	16·86	46·87	2·46	66·19	7·86	22·77	63·30	3·32	10·61	0·60
	98th	82·53	24·65	50·56	2·05	77·26	5·27	29·87	61·26	2·48	6·39	*
Normal sow 7 S 1	11th	82·82	14·70	55·63	2·15	72·48	10·34	17·75	67·17	2·60	12·48	*
	39th	82·99	16·13	51·20	3·09	70·42	12·57	19·43	61·69	3·72	15·16	0·50
	70th	84·00	19·31	47·40	3·95	70·66	13·34	22·99	56·43	4·70	15·88	1·00
	90th	83·80	20·88	40·24	2·25	63·37	20·43	24·92	48·01	2·69	24·38	0·80
Normal sow 10 S 2	56th	83·01	15·95	51·26	1·56	68·77	14·24	19·22	61·75	1·88	17·15	1·30
	98th	82·68	21·75	46·88	1·55	70·18	12·50	26·31	56·69	1·88	15·12	1·00
Ca-def. sow 77 N 8	12th	83·91	14·28	61·66	1·87	77·81	6·10	17·02	73·47	2·23	7·28	1·20
	52nd	72·60	17·76	45·81	1·92	65·49	7·11	24·46	63·10	2·64	9·80	1·10
	85th	84·99	16·41	56·58	2·13	75·12	9·87	19·31	66·56	2·51	11·62	1·60
	87th	71·63	14·76	47·18	2·41	64·34	7·29	20·61	65·86	3·36	10·17	Same
	108th	78·15	17·28	39·52	1·42	58·22	19·93	22·11	50·56	1·82	25·51	0·52
Ca-def. sow 32 p 3	14th	83·80	18·20	56·92	1·69	76·81	6·99	21·72	67·93	2·01	8·34	1·32
	31st	72·58	20·15	38·60	1·45	60·20	12·38	27·76	53·19	2·00	17·06	1·90
	73rd	73·49	21·21	48·11	2·35	71·67	1·82	28·86	65·46	3·20	2·48	0·30
	77th	78·25	19·63	47·03	2·51	69·17	9·08	25·09	60·10	3·21	11·61	0·30
	95th	85·39	21·11	52·46	2·51	76·08	9·31	24·72	61·44	2·94	10·90	2·10

* Not weighed.

The nitrogen retention fluctuates to some extent with the live weight increase recorded during the period, inasmuch as the balance tends to be high when the live weight increase is favourable. A cursory examination of the figures, however, shows that there is no direct correlation between these two factors. This has been noted by several workers on balance experiments and renders the interpretation of results more difficult. The outstanding nature of some of the figures, however, together with the general trend of the results enables certain deductions to be made with confidence.

As was pointed out in the introduction, the conclusions of different workers on the retention of nitrogen during the first half of gestation are very conflicting. Thus, while in some cases a short period of active retention has been observed followed by a negative balance or nitrogen equilibrium, in other experiments the initial stages of pregnancy were marked by a negative nitrogen balance, the storage of protein occurring only in the second half of gestation. With the object of further investigating this point, four balances were taken with four sows immediately after service and also about the fifth week of gestation (39th to 52nd day).

The results show that it was only in the case of 8 N 2 that pronounced nitrogen retention occurred immediately after service, the balance results for 7 S 1 and 77 N 8 being the lowest recorded for these sows, while that of 32 p 3 was the lowest but one. That high nitrogen retention took place in the case of 8 N 2 is confirmed by the analysis of three separate composite samples of faeces and urine, the balances being 13·91, 16·70 and 17·54 gm. of nitrogen respectively. This inconsistency is probably due to the fact that the first period with 8 N 2 was taken very soon after the pigs were weaned, while in the case of the other sows, since the litters died immediately after birth, there was no lactation period. It is reasonable to suppose that the drainage imposed on the maternal tissues by milk secretion would be followed by a period of recuperation and active storage. The other three sows, after farrowing, were allowed to recuperate before the balance was taken and this enabled them to commence pregnancy with a reserve of nitrogen stored up in their tissues. The figures show that the first half of pregnancy can be a period of steady nitrogen retention. With the exception of 8 N 2, the sows show a definite increase in protein storage between the 39th and 52nd days of gestation compared with the initial stages. Murlin(5), in his work on pregnant bitches, has attributed the negative nitrogen balances that he observed in the first half of pregnancy to the destruction of maternal protoplasm

which he considers necessary in order to provide the developing foetus with the proper *type* of protein. This indicates that the type of protein incorporated in the rations of these sows is of high metabolic efficiency for the purpose of reproduction and was supplied in sufficient amount to protect the mother organism or to rebuild any destruction taking place in converting maternal to foetal protein.

It is generally agreed by workers on pregnant animals that marked conservation of protein takes place only within a few weeks of parturition. Thus Murlin, in the work already referred to, found that in pregnant bitches marked retention only occurred during the last two weeks, while Crowther and Woodman, in their "Study of Nitrogen Metabolism in the Dairy Cow," observed that the rate of storage was not pronounced until within three or four weeks of parturition. It will be interesting therefore to compare these findings with the results on pregnant sows given in Table IV.

The pronounced conservation of nitrogen by 7 S 1 and 77 N 8 on the 90th and 108th days of pregnancy is noteworthy and shows that a special demand for protein occurs at this period. In the case of 77 N 8 the nitrogen retention was doubled on the 108th day compared with the 85th and 87th days, while 7 S 1, although retaining high amounts of protein on the 70th day, yet retained a daily average of 7 gm. more of nitrogen on the 90th day. The figures show that in both cases this was due to a decrease in the urinary excretion of nitrogen. On the other hand, 10 S 2 and 32 p 3 showed no increased retention up to the 98th and 95th days respectively. A high retention of nitrogen towards parturition is what would be expected since the mammary glands at this period show active development in preparation for lactation.

It will be interesting here to compute the amount of nitrogen stored during gestation and also the amount deposited in the foetus. The average daily retention of nitrogen in nine periods taken with normal sows was 12·51 gm. The sum-total retention for 115 days' gestation was therefore 1439 gm. nitrogen. The average weight of 62 normal pigs, at birth, was 2·57 lb. or 1167 gm. According to Wilson (16), a young pig at birth contains from 11·9 to 12·5 per cent. protein, so we can assume a protein content of 12·5 per cent. or 2 gm. N per 100 gm. The approximate nitrogen content of these normal pigs would be therefore (11·67 gm. $\times$ 2·0) 23·34 gm. and taking the average litter to be 10 pigs the storage in the foetus becomes 233 gm. N. Thus, out of a retention of 1439 gm. of nitrogen the actual storage in the foetus, without adnexa, was only 233 gm. Even after allowing for the loss of nitrogen in the placental

fluids and adnexa as well as for the development of the mammary glands, etc., it seems that a considerable reserve was stored in the animal organism in preparation for lactation. The storage was six times as much as was required for actual deposition in the foetus.

The average daily retention of nitrogen in 10 periods with the calcium-deficient group was 8·99 gm. and for 115 days 1034 gm. The third period with 32 p 3, however, gave an abnormally low balance, and in view of the retention recorded on the 77th day of pregnancy it seems advisable to discard this period. The average retention then becomes 9·78 gm. and the total nitrogen storage throughout gestation 1125 gm. The average weight of 52 calcium-deficient pigs was 2·64 lb., which is equivalent to 1199 gm. Estimating the nitrogen content at 2·0 per cent, as before, the total storage per pig becomes 23·98 gm. nitrogen and for 10 pigs, which was the average litter, 239·8 gm. Therefore, out of a sum-total storage of 1125 gm. of nitrogen only 239·8 gm. were deposited in the foetus, again showing a retention of nitrogen almost five times as much as the foetal requirement. These conclusions are in excellent agreement with those of Wilson (9), who worked on three human pregnancies. He observes that "Storage of nitrogen begins at a much earlier period than has hitherto been supposed possible and the organism may acquire the capacity of storing nitrogen from the very beginning of pregnancy." Storage continues throughout the entire duration of pregnancy and is greatly in excess of the actual needs of the developing ovum. A large proportion of the nitrogen stored is added to the general maternal organism as "rest-material."

The important point, however, is that excessive nitrogen storage occurred in the calcium-deficient sows as well as in the normal group and the question arises "Did calcium deficiency retard protein metabolism?" The nitrogen retention in the case of the normal sows was 1439 gm. and in the calcium-deficient 1125 gm. Although these figures are only rough approximations, yet they seem to show definitely that there is a relationship between the supply of lime in the food and the retention of protein.

THE AVERAGE DAILY ASH BALANCES.

The average daily ash balances are given in Table V. It will be observed that the ash intake of the normal group of sows was considerably higher than that of the calcium-deficient group, this being due to the addition of calcium carbonate to the ration of the normal sows.

The retention of ash was positive in each period, but was considerably

Table V. *Showing the daily ash balances and the percentage of the ash intake excreted and retained.*

Experimental no. of sow	Day of gestation	Intake of ash in gm.	Gm. of ash excreted in faeces	Gm. of ash excreted in urine	Gm. of ash excreted in washings	Total gm. of ash excreted	Total gm. of ash retained	% of ash intake in faeces	% of ash intake in urine	% of ash intake in washings	% of ash intake retained	Live weight increase per day (lb.)
Normal sow 8 N 2	13th	121·87	63·05	30·27	2·25	95·57	26·30	51·74	24·86	1·87	21·53	1·40
	49th	128·91	65·95	31·57	3·99	101·51	27·40	51·16	24·49	3·10	21·25	0·60
	98th	117·60	69·71	29·28	0·297	99·29	18·31	59·26	24·90	0·25	15·57	—
Normal sow 7 S 1	11th	116·42	58·59	33·30	2·86	94·75	21·67	50·33	28·60	2·45	18·62	—
	39th	117·93	47·40	30·66	4·30	82·36	35·57	40·20	26·00	3·64	30·16	0·50
	70th	120·06	56·92	32·44	5·15	94·51	25·55	47·41	27·02	4·29	21·28	1·00
	90th	120·96	57·12	27·44	3·567	83·13	32·83	47·22	22·69	2·96	27·13	0·80
Normal sow 10 S 2	56th	120·73	59·47	26·45	2·67	88·59	32·14	49·27	21·91	2·20	26·62	1·30
	98th	116·15	68·85	34·40	2·08	105·33	10·82	59·28	29·62	1·79	9·31	1·00
Ca-def. sow 77 N 8	12th	75·40	39·77	30·09	3·15	73·01	2·39	52·75	39·90	4·18	3·17	1·20
	52nd	77·11	36·33	30·29	2·77	69·39	7·72	47·12	39·27	3·59	10·02	1·10
	85th	91·39	40·53	35·52	2·99	79·04	12·35	44·36	38·86	3·27	13·51	1·60
	87th	77·60	35·80	32·56	3·27	71·63	5·97	46·13	41·96	4·20	7·69	Same
	108th	84·65	49·20	22·96	3·167	75·33	9·32	58·12	27·13	3·74	11·01	0·52
Ca-def. sow 32 p 2	14th	75·03	35·48	32·764	2·634	70·88	4·15	47·29	43·66	3·52	5·53	1·32
	31st	76·17	34·06	29·58	2·730	66·37	9·79	44·73	38·84	3·58	12·85	1·90
	73rd	78·51	39·58	34·60	2·720	76·90	1·61	50·37	44·09	3·48	2·06	0·30
	77th	83·77	37·03	34·95	3·69	75·67	8·10	44·20	41·73	4·40	9·67	0·30
	95th	91·80	43·71	31·83	2·29	77·83	13·97	47·61	34·66	2·50	15·23	2·10

higher in the high-calcium group, this indicating a distinct utilisation of lime by these sows. It is noteworthy that while the faecal excretion of ash was considerably lower on the calcium-deficient ration, yet the percentage of the intake excreted through the intestine was very similar in the two groups. The urinary excretion of ash, however, shows a more economical utilisation of minerals by the calcium-deficient sows, the percentage of the intake excreted in the urine being considerably higher.

The results do not show any increased conservation of ash with the advancement of pregnancy, but indicate a steady retention throughout gestation. This, however, will be dealt with more fully in discussing the individual balances of the different minerals.

The average daily lime balances.

The average daily retention of lime in both groups of sows is given in Table VI.

1. *The average daily lime balance in the normal group.*

A surprising result brought out by the figures in Table VI is that, with the exception of the first balance taken with 7 S 1, the advancement of gestation is accompanied by a *decrease* in the amount of lime retained. This is contrary to what would be expected since, in the second half of pregnancy, the deposition of lime in the skeleton of the young foetus should lead to an increased demand on the calcium ingested in the ration and should therefore stimulate the retention of lime. A similar abnormality in lime metabolism has been commented upon by other investigators. Thus, Hart (17) and his associates, working with a dairy cow, found a persistent excretion of 22 gm. of CaO in the faeces on an intake of 24 gm. of lime, while at the same time 18 gm. of calcium oxide was given out daily in her milk. Similarly Forbes (18) and his co-workers, in their investigations on mineral metabolism in the milch cow, observed a persistent negative lime balance even when the ration was rich in lime. Do these results indicate that when the demand for lime is heavy, the animal finds it easier to make use of skeletal reserves of lime rather than metabolise the lime ingested in the food, or is there a maximum limit to the amount of lime that can be reserved so that by active storage in the initial stages or first half of pregnancy the animal is able to tide over the latter part of gestation and towards parturition retain only the minimum amount of lime necessary? If the former is true, it shows the importance of feeding lime to pregnant animals in the first half of gestation, otherwise only a small proportion will become available for foetal

Table VI. *Showing the daily lime balances and the percentage of the lime intake excreted and retained.*

Experimental no. of sow	Day of gestation	Intake of CaO in gm.	Gm. CaO excreted in faeces	Gm. CaO excreted in urine	Gm. CaO excreted in washings	Total gm. of CaO excreted	Total gm. of CaO retained	% of CaO intake in faeces	% of CaO intake in urine	% of CaO intake in washings	% of CaO intake retained	Live weight increase per day (lb.)
Normal sow 8 N 2	13th	27·06	17·44	1·121	0·153	18·71	8·35	64·45	4·14	0·56	30·85	1·40
	49th	26·50	20·08	0·532	0·222	20·83	5·67	75·76	2·01	0·83	21·40	0·60
	98th	26·52	22·35	0·974	0·228	23·55	2·97	84·28	3·66	0·86	11·20	—
Normal sow 7 S 1	11th	26·31	21·37	1·235	0·227	22·83	3·48	81·20	4·72	0·86	13·22	—
	39th	26·54	14·62	0·747	0·350	15·72	10·82	55·09	2·81	1·32	40·78	0·50
	70th	26·38	16·62	0·669	0·441	17·73	8·65	62·99	2·54	1·68	32·79	1·00
	90th	26·40	16·94	1·251	0·253	18·44	7·96	64·17	4·73	0·95	30·15	0·80
Normal sow 10 S 2	56th	26·47	18·74	0·480	0·290	19·51	6·96	70·79	1·81	1·10	26·30	1·30
	98th	26·30	20·85	2·772	0·166	23·79	2·51	79·29	10·54	0·63	9·54	1·00
Ca-def. sow 77 N 8	12th	1·667	1·103	0·211	0·133	1·447	0·220	66·16	12·66	7·98	13·20	1·20
	52nd	1·284	1·240	0·213	0·115	1·568	−0·284	96·56	16·59	8·95	−22·10	1·10
	85th	2·026	1·397	0·247	0·143	1·787	0·239	68·96	12·18	7·06	11·80	1·60
	87th	1·783	1·125	0·184	0·102	1·411	0·372	63·10	10·32	5·72	20·86	Same
	108th	1·945	1·147	0·206	0·175	1·528	0·417	58·97	10·59	9·00	21·44	0·52
Ca-def. sow 32 p 3	14th	1·655	1·373	0·294	0·134	1·801	−0·146	82·96	17·76	8·10	− 8·82	1·32
	31st	1·290	1·190	0·260	0·140	1·590	−0·300	92·23	20·17	10·86	−23·26	1·90
	73rd	1·226	1·022	0·222	0·074	1·318	−0·092	83·35	18·10	6·03	− 7·48	0·30
	77th	1·920	1·013	0·153	0·160	1·326	0·594	52·74	7·98	8·33	30·95	0·30
	95th	2·338	1·263	0·180	0·086	1·529	0·809	54·02	7·70	3·69	34·59	2·10

nutrition. The fact that between 30 and 40 per cent. of the intake was retained by these sows at four different periods suggests that the lime-phosphate ratio in the food was not the controlling factor in retention.

It will be observed that the excretion of lime took place mainly through the wall of the intestine. At the commencement and end of pregnancy, however, the urine is distinctly richer in lime than in the intermediate stages of gestation. This will be discussed in greater detail, however, when dealing with the inter-relationship between lime and phosphate metabolism.

2. *The average daily retention of lime in the calcium-deficient group.*

Studies in animal nutrition have shown that an animal, when placed on a ration containing protein in excess of its requirement, will, after an initial period of storage, accommodate itself to the ration and in time attain a higher plane of nutrition when rough equilibrium between intake and output prevails. The balance results for the calcium-deficient pigs show that the same phenomenon takes place in mineral metabolism and also that the converse of the above statement is also true, namely, that the animal can adjust itself to a plane of nutrition much lower than the optimum. When investigating the maintenance requirement for lime or any other constituent it has been usual in the past to determine the balance between intake and output until the minimum amount which will permit of the maintenance of equilibrium is found. Is it possible, "provided sufficient time is allowed," that an animal can attain equilibrium on an intake much lower than the true maintenance requirement? The surprising feature of these results is that these pregnant sows, on a daily intake of less than 2 gm. CaO, in four gestation periods, appear to have established a condition of approximate lime equilibrium. While it is true that in four periods negative lime balances are recorded, which, calculated in percentage of the small lime intake, seem fairly pronounced, yet it will be observed that the intake of calcium in the food at these periods has also dropped into a fairly low level. When the intake in the food approaches 2 gm. CaO, these deficient sows even show slight storage. Keeping in mind the experimental error that is liable to occur in this type of work, one is forced to the conclusion that these sows were at approximate equilibrium. This illustrates the remarkable physiological capacity of the organism to economise or adjust itself and make the maximum use of any mineral element deficient in an otherwise satisfactory ration. It appears that the animal is able to reduce the lime losses from her body to a minimal figure, thus devoting the lime from

her own bones and tissues for the development of the foetus. As already stated, the live weight of the calcium-deficient piglings at birth was quite normal which suggests that it was the maternal organism that suffered and not the offspring. The results show further that economy in lime metabolism becomes enhanced as gestation advances since in both the deficient animals increased lime retention is distinctly observed in the last two periods.

A rough approximation of the amount of lime that the calcium-deficient sows had to provide from their own organism, and a measure of the damage suffered on a cereal ration can be estimated from the following data. In the ten periods taken, the average daily intake of calcium oxide was 1·713 gm. Taking the period of gestation as 115 days, the total intake throughout pregnancy was 197 gm. CaO. The average daily excretion of lime in the ten periods was 1·530 gm. or a total of 176 gm. CaO for the whole gestation period. This indicates a storage of 21 gm. CaO for foetal development, hypertrophy of the uterus and glandular tissues connected with reproduction, as well as for the development of the placental membranes and fluids.

In 1914 Hart and his co-workers published data on the amount of lime in the skeleton of young pigs at birth. The pregnant animals in their experiment were confined to wooden floors and one lot was given a ration designed to supply 1·56 gm. CaO and 21·4 gm. P_2O_5 per day throughout gestation. These records of intake agree well with those fed to the deficient animals in this experiment, the only difference being that in Hart's work the sows after farrowing were turned out to pasture until the experiment was resumed the following year, and therefore, presumably, had a skeletal reserve of lime, whereas these sows were in the second generation of calcium deficiency. Hart selected a representative animal from each of five litters and the amount of lime in the skeleton at birth was determined. The minimum amount of lime in the skeleton was 11·4 gm., the maximum on the same ration being 18·5 gm. CaO.

Taking the minimum figure given by Hart as being the amount of lime deposited in the skeletons of these calcium-deficient piglings, and multiplying this by 10, the average number of normal pigs farrowed by the calcium-deficient group throughout the whole period of the experiment, the total deposition of lime in the skeletons of the young foetuses amounts to 114 gm. CaO. Further, the minimum amount of lime in the soft tissues of the foetus at birth was found to be 0·5 gm. so that in a litter of 10 pigs this would amount to 5 gm. We therefore arrive at an approximate minimal figure of 119 gm. CaO for the foetus only. This

figure, minus the storage of 21 gm. CaO previously arrived at from the balance results, shows that the calcium-deficient sows had to sacrifice at least 100 gm. of CaO from their own organism to supply the needs of the foetus only.

Hart also analysed the skeletons of 14 pigs when the daily CaO intake of the mother was between 8·2 and 8·6 gm. The average figure he arrived at for the amount of lime in the skeleton was 16·3 gm. Further, the maximum figure he obtained for the lime in the soft tissues was 1·20 gm. From these figures it appears therefore that the deposit of lime in the foetus alone in normal pregnancy amounts to 175 gm. Taking this figure as a basis, the calcium-deficient sows in this experiment had to supply a deficiency of 154 gm. CaO for foetal development.

The average daily lime balance from nine periods with the normal sows in this experiment was 6·40 gm. CaO. During the 115 days of gestation these sows, therefore, utilised 736 gm. CaO. This figure represents a storage of nearly five times the amount deposited in the foetus during normal pregnancy and stands out in striking contrast to the storage of 21 gm. computed for the calcium-deficient group. The fact that the deficient sows had no milk when they farrowed, and moreover had great difficulty in farrowing, needs no further explanation; that they became lame during pregnancy and showed symptoms similar to rickets is not surprising. The data given supports the conclusions of other workers that pregnancy is a preparation for parturition and lactation and does not necessarily involve a sacrifice by the mother organism.

THE AVERAGE DAILY PHOSPHATE BALANCES.

The results showing the average daily phosphate balances and the percentage of the intake excreted and retained are given in Table VII.

Although the conservation of phosphate varied considerably, it will be observed that retention occurred at all stages of gestation. The highest balance for 8 N 2 was recorded immediately after service, but in 7 S 1, 77 N 8 and 32 p 3 this did not occur until the fifth or sixth week of pregnancy. The results demonstrate that the retention of phosphate is as active in the first half of gestation as it is in the second half and there is no indication that marked conservation takes place with the approach of parturition as was observed to be the case with the nitrogen balances. This result is therefore contradictory to that of Ver Eeke and others referred to in the introduction, but is in agreement with the results of Hoffstrom on human pregnancy. In the normal sows, this abnormality may be correlated with the fact already mentioned that the lime retention

Table VII. *Showing the daily phosphate balances and the percentage of the intake excreted and retained.*

Experimental no. of sow	Day of gestation	Intake of P_2O_5 in gm.	Gm. P_2O_5 excreted in faeces	Gm. P_2O_5 excreted in urine	Gm. P_2O_5 excreted in washings	Total gm. of P_2O_5 excreted	Total gm. of P_2O_5 retained	% of P_2O_5 intake in faeces	% of P_2O_5 intake in urine	% of P_2O_5 intake in washings	% of P_2O_5 intake retained	Live weight increase per day (lb.)
Normal sow 8 N 2	13th	24·49	16·56	0·269	0·129	16·96	7·53	67·63	1·10	0·52	30·75	1·40
	49th	25·15	18·04	2·804	0·402	21·25	3·90	71·72	11·15	1·60	15·52	0·60
	98th	22·34	17·96	0·422	0·124	18·51	3·83	80·41	1·89	0·55	17·14	—
Normal sow 7 S 1	11th	20·95	15·12	0·919	0·165	16·20	4·75	72·17	4·38	0·77	22·68	—
	39th	22·46	12·55	0·250	0·218	13·02	9·44	55·88	1·11	0·97	42·04	0·50
	70th	22·52	14·39	0·112	0·250	14·75	7·77	63·89	0·50	1·12	34·49	1·00
	90th	22·90	14·26	0·090	0·133	14·48	8·42	62·26	0·39	0·58	36·77	0·80
Normal sow 10 S 2	56th	22·97	15·85	0·460	0·100	16·41	6·56	69·01	2·00	0·43	28·56	1·30
	98th	20·84	16·47	0·104	0·074	16·65	4·19	79·03	0·50	0·36	20·11	1·00
Ca-def. sow 77 N 8	12th	21·24	8·450	10·438	0·302	19·19	2·05	39·78	49·15	1·42	9·65	1·20
	52nd	27·27	9·700	6·410	0·450	16·56	10·71	35·57	23·51	1·65	39·27	1·10
	85th	31·32	11·655	9·130	0·531	21·32	10·00	37·24	29·15	1·68	31·93	1·60
	87th	22·17	8·030	10·590	0·470	19·09	3·08	36·22	47·76	2·12	13·90	Same
	108th	24·18	9·902	8·102	0·265	18·27	5·91	40·95	33·52	1·09	24·44	0·52
Ca-def. sow 32 p 3	14th	21·14	9·442	10·140	0·320	19·90	1·24	44·67	47·96	1·50	5·87	1·32
	31st	27·17	9·180	5·820	0·380	15·38	11·78	33·79	21·43	1·41	43·37	1·90
	73rd	27·91	10·410	9·960	0·595	20·97	6·94	37·30	35·70	2·13	24·87	0·30
	77th	23·95	9·181	12·180	0·532	21·89	2·06	38·32	50·86	2·21	8·61	0·30
	95th	31·44	13·030	15·340	0·598	28·97	2·47	41·45	48·79	1·90	7·86	2·10

decreased in the second half of pregnancy and if the skeletal reserve of calcium was drawn upon for the purpose of calcification in the foetus, then phosphate would also enter into the metabolic field and supply some of the demand which would otherwise have been ingested from the ration.

The phosphate retention of the calcium-deficient sows is not only positive in all cases, but also the conservation observed in three periods is higher than any recorded for the normal group. Further, it is noteworthy that in both of the deficient sows the highest phosphate retention coincides with the heaviest negative lime balances, when the percentage of the lime intake retained was lowest. Whether the phosphate retention over the whole period of gestation is enhanced by the lime content of the food will, however, be discussed below.

The path of phosphate elimination shows a distinct difference in the metabolism of this element in the two groups of sows. When lime is abundant in the food the excretion takes place mainly through the wall of the intestine, but in the absence of lime a pronounced increase in urinary phosphate is observed, excretion taking place *via* the kidney as soluble sodium and potassium salts. A significant fact demonstrated in Table VII is the high degree of phosphate absorption that takes place in the absence of lime. The figures show that at least from 55 to 66 per cent. of the intake was absorbed into the blood by the calcium-deficient group which clearly demonstrates that lime is not necessarily a factor in phosphate absorption. On the other hand, it may be that this high concentration of phosphate in the blood is an important factor in lime economy, since it is possible, as observed by Theiler (19), "that this high concentration may have a physiological affinity and tend to draw out the maximum amount of calcium from the food." The figures indicate that in the absence of calcium, phosphate retention takes place in some other combination.

The average daily retention of phosphate in the normal group of sows was 6·26 gm. P_2O_5, which represents a total conservation of 720 gm. P_2O_5 throughout the whole period of gestation. In the lime-deficient group the average retention in 10 periods was 5·62 gm., so that during the 115 days of gestation a storage of 647 gm. P_2O_5 took place. Although the storage of phosphate by the lime-deficient animals must have been considerably in excess of the requirements of the foetus, yet it will be observed that it was 73 gm. P_2O_5 lower than occurred in the high-calcium group.

THE AVERAGE DAILY POTASH BALANCES.

The figures showing the average daily retention of potash and the percentage of the intake excreted and retained by the two groups of sows are given in Table VIII. It will be noted that retention occurred at all stages of gestation, as was observed to be the case with the other elements, though the actual balance is considerably smaller than was found for the lime and phosphate.

In 8 N 2 the highest conservation of potash took place at the commencement of gestation, while in the other three sows comparatively high balances were obtained about the sixth week. It is significant that in 7 S 1 and 77 N 8 a marked increased retention took place within a month of parturition, this behaviour being analogous to that of the protein balance. The retention of 32 p 3 on the 90th day was also distinctly higher than in the intermediate stages. This utilisation of potash towards parturition is undoubtedly connected with the formation of new blood and the manufacture of red-blood corpuscles.

It is noteworthy that the two groups of sows show a distinct difference in the path of potash excretion. In the high-calcium group, from 45 to 67 per cent. of the intake was eliminated in the urine, but when lime was deficient in the ration an increased excretion of potash through the intestine occurred so that the amount of excretion was on the whole fairly equally divided between the urine and faeces. Undoubtedly, in the absence of lime, potash plays a rôle in phosphate excretion since the elimination of acid phosphate, as mono- or dihydrogen salt, has been observed by many investigators to be a mode of protective mechanism of the body in acidosis.

The average daily retention of potash in the nine balances taken with the normal sows was 1·745 gm. K_2O. This represents a total conservation of 201 gm. throughout the period of gestation. In the calcium-deficient group the average daily retention was 0·963 gm. K_2O so that the total retention for 115 days becomes 111 gm. K_2O.

It will be interesting to compare these findings with the analytical results for the mineral composition of the bodies of pregnant gilts published by Griswold and others (20). According to these workers the percentage ash in the entire body of a pregnant gilt (based on empty weight) is approximately 2·2, of which 9·1 per cent. is K_2O. This is equivalent to 0·20 per cent. K_2O in the entire body, a figure almost identical with that of Lawes and Gilbert for the store pig.

From the live weight data given (p. 765) it will be observed that

Table VIII. *Showing the average daily potash balances and the percentage of potash excreted and retained.*

Experimental no. of sow	Day of gestation	Intake of K_2O in gm.	Gm. K_2O excreted in faeces	Gm. K_2O excreted in urine	Gm. K_2O excreted in washings	Total gm. of K_2O excreted	Total gm. of K_2O retained	% of K_2O intake in faeces	% of K_2O intake in urine	% of K_2O intake in washings	% of K_2O intake retained	Live weight increase per day (lb.)
Normal sow 8 N 2	13th	15·04	4·190	7·373	0·609	12·17	2·87	27·85	49·03	4·04	19·08	1·40
	49th	17·74	4·210	10·030	0·850	15·09	2·65	23·74	56·53	4·79	14·94	0·60
	98th	14·76	5·172	7·066	0·633	12·87	1·89	35·04	47·87	4·29	12·80	—
Normal sow 7 S 1	11th	15·20	4·030	9·449	0·782	14·26	0·94	26·51	62·18	5·14	6·17	—
	39th	14·83	3·655	9·018	0·930	13·60	1·23	24·64	60·80	6·27	8·29	0·50
	70th	16·67	4·810	9·365	1·155	15·33	1·34	28·85	56·18	6·93	8·04	1·00
	90th	16·77	5·320	8·105	0·931	14·35	2·41	31·74	48·34	5·55	14·37	0·80
Normal sow 10 S 2	56th	16·57	4·690	9·200	0·970	14·86	1·71	28·31	55·52	5·85	10·32	1·30
	98th	15·12	4·122	9·819	0·507	14·45	0·67	27·27	64·95	3·35	4·43	1·00
Ca-def. sow 77 N 8	12th	15·39	6·984	7·040	0·738	14·76	0·629	45·37	45·75	4·79	4·09	1·20
	52nd	16·46	6·700	7·300	0·780	14·78	1·680	40·70	44·35	4·74	10·21	1·10
	85th	18·77	7·950	8·858	0·537	17·35	1·42	42·36	47·21	2·86	7·57	1·60
	87th	15·27	6·572	7·262	0·891	14·73	0·54	43·05	47·56	5·84	3·54	Same
	108th	16·66	8·913	4·768	0·586	14·27	2·39	53·51	28·63	3·52	14·34	0·52
Ca-def. sow 32 p 3	14th	15·30	6·836	7·362	0·618	14·816	0·484	44·68	48·12	4·04	3·16	1·32
	31st	16·43	7·280	7·130	0·750	15·160	1·27	44·31	43·40	4·56	7·73	1·90
	73rd	16·77	7·580	8·380	0·685	16·65	0·12	45·22	49·97	4·09	0·72	0·30
	77th	16·57	6·808	8·846	0·822	16·476	0·096	41·09	53·39	4·95	0·57	0·30
	95th	18·86	9·55	7·580	0·744	17·87	0·99	50·63	40·19	3·94	5·24	2·10

normal sow 7 S 1 increased in weight at the rate of 1·51 lb. per day and her average K_2O retention in the corresponding three periods was 1·66 gm. per day. Expressing this as a percentage of the live weight increase the figure obtained is 0·24 per cent. K_2O, which is in good agreement with the results mentioned above. The average live weight increase per day for the three normal sows was 1·46 lb. (663 gm.) and calculating on the average daily retention of 1·745 gm. K_2O, the percentage potash in the live weight increase becomes 0·26, a figure slightly higher than that obtained by actual analysis which is based on the total weight, including the skeleton, which contains very little potash.

From similar data given by Griswold, it can be calculated that the foetus of 7 S 1 contained 32·3 gm. K_2O, and the 133 lb. live weight increase (p. 764) on the basis of 0·20 per cent. K_2O would contain 121 gm. K_2O, making a total of 153 gm. K_2O out of a total retention of 170 gm. during gestation, the 17 gm. difference being present in the placenta and fluids.

It should be noted, however, that the retention of K_2O per cent. of the live weight increase was distinctly lower in the calcium-deficient group than in the normal sows. On the basis of 1·46 lb. increase in weight and a K_2O retention of 0·963 gm. per day, the percentage becomes 0·14 compared with 0·25 for the normal group. This will be discussed in dealing with the sodium balances.

The influence of calcium deficiency on the blood.

The function of potash in the formation of red-blood corpuscles and the fact that indications of anaemia had been observed in some of the young piglings of the calcium-deficient sows suggested that it would be interesting to examine the blood of these two groups of sows. This was done, with the co-operation of Mr A. C. Fraser of the School of Animal Pathology, with the following results:

	Cell counts	
	Red corpuscles	Leucocytes per c.mm.
Calcium-deficient sow 77 N 8	5,512,000	15,000
	6,840,000	14,340
Calcium-deficient sow 32 p 3	6,080,000	9,400
	6,400,000	14,000
	6,656,000	9,500
	6,784,000	8,900
Normal sow 8 N 2	8,160,000	8,000
	8,224,000	10,200
	8,192,000	10,000

The differential leucocytic count was as follows:

	Lymphocytes	Monocytes	Polymorphoneuclears	Eosinophiles	Basophiles
Ca-def. sow 77 N 8 ...	9027	841	4590	535	306
Ca-def. sow 77 N 8 ...	9009	358	3713	926	236
Ca-def. sow 32 p 3 ...	6298	282	1786	846	138
Ca-def. sow 32 p 3 ...	9940	280	3080	644	56
Complete-ration sow 8 N 2	4387	400	4663	420	130

The red cells show a deficit of 2,000,000 in 77 N 8 and of 1,700,000 in 32 p 3 as compared with the control pig, which indicates a definite though not serious anaemia. The haemoglobin percentage showed a slightly lower figure for the deficient pigs, but not more than would be accounted for by the difference in the number of red cells. The appearance of the cells both in fresh and stained specimens indicated no deficiency in haemoglobin on the part of individual cells. The differential count also showed a slight but consistent lymphocytosis in both the deficient pigs. This is interesting since lymphocytosis has been associated with anaemia in human beings. The calcium content of the blood in the deficient pigs was 6·3 mgm. per 100 c.c., while in the normal pigs it was 11·8 mgm. CaO.

The above observations suggest that the depressing influence of lime deficiency on the retention of potash has resulted in a lower utilisation of iron, and slight symptoms of anaemia, even though the ration contained blood meal which is rich in iron. The important conclusion arrived at, therefore, is that although a mineral ingredient may be present in the ration in excessive amounts, yet its proper utilisation depends on the proportion of the other mineral ingredients present in the food.

The average daily soda balances in the two groups of sows.

The average daily soda balances and the percentage of the intake excreted and retained are given in Table IX.

The figures show considerable variation in the amount of soda retained in the different periods, but it was only in the first period with 77 N 8 that a slight negative balance was obtained. All the sows with the exception of 8 N 2 show very small retention of soda in the initial stages of gestation, but by the seventh week of pregnancy there is a distinct increase in the rate of storage in all the pigs worked with.

The highest conservation of soda in the case of four sows occurred within a month of parturition, the storage of the two calcium-deficient sows especially being particularly high on the 95th and 108th days of gestation.

Table IX. *Showing the average daily soda* (Na_2O) *balances and the percentage of the intake excreted and retained.*

Experimental no. of sow	Day of gestation	Intake of Na_2O in gm.	Gm. Na_2O excreted in faeces	Gm. Na_2O excreted in urine	Gm. Na_2O excreted in washings	Total gm. of Na_2O excreted	Total gm. of Na_2O retained	% of Na_2O intake in faeces	% of Na_2O intake in urine	% of Na_2O intake in washings	% of Na_2O intake retained	Live weight increase per day (lb.)
Normal sow 8 N 2	13th	9·670	1·676	5·605	0·450	7·730	1·94	17·33	57·97	4·65	20·05	1·40
	49th	9·800	1·950	5·840	0·469	8·260	1·54	19·90	59·60	4·79	15·71	0·60
	98th	10·387	2·227	6·383	0·470	9·080	1·31	21·44	61·45	4·52	12·61	—
Normal sow 7 S 1	11th	10·158	1·967	7·388	0·532	9·887	0·271	19·37	72·75	5·22	2·66	—
	39th	10·40	1·542	6·813	0·658	9·010	1·390	14·82	65·50	6·32	13·36	0·50
	70th	9·590	1·747	5·110	1·333	8·190	1·400	18·22	53·28	13·90	14·60	1·00
	90th	9·607	3·058	4·354	0·570	7·982	1·630	31·82	45·31	5·92	16·95	0·80
Normal sow 10 S 2	56th	9·668	1·620	6·450	0·530	8·60	1·07	16·75	66·70	5·48	11·07	1·30
	98th	10·17	3·206	5·361	0·237	8·804	1·366	31·52	52·72	2·32	13·44	1·00
Ca-def. sow 77 N 8	12th	11·249	1·709	9·032	0·687	11·428	−0·180	15·19	80·30	6·11	−1·60	1·20
	52nd	11·570	1·143	7·220	0·550	8·910	2·66	9·87	62·40	4·75	22·98	1·10
	85th	12·604	1·910	7·348	0·631	9·889	2·72	15·13	58·31	5·00	21·56	1·60
	87th	10·445	1·790	7·936	0·533	10·259	0·186	17·14	75·98	5·10	1·78	Same
	108th	11·395	1·219	5·147	0·535	6·901	4·494	10·69	45·17	4·70	39·44	0·52
Ca-def. sow 32 p 3	14th	11·377	2·334	8·064	0·437	10·835	0·542	20·50	70·91	3·83	4·76	1·32
	31st	11·200	2·130	6·750	0·650	9·530	1·67	19·02	60·27	5·80	14·91	1·90
	73rd	11·220	3·150	6·660	0·513	10·320	0·900	28·06	59·36	4·56	8·02	0·30
	77th	11·366	2·561	7·285	0·588	10·434	0·932	22·53	64·09	5·18	8·20	0·30
	95th	12·760	1·920	6·790	0·336	9·040	3·720	15·03	53·20	2·63	29·14	2·10

The path of excretion of sodium is very similar in both groups, from 45 to 80 per cent. of the intake being recovered in the urine. The deficiency of lime had therefore a greater effect on the path of potash excretion than on the soda.

The average daily retention of soda by the normal group of sows was 1·324 gm. Na_2O, the total conservation throughout gestation being, therefore, 152 gm. The average balance from 10 periods with the calcium-deficient sows was 1·764 gm. representing a total storage in 115 days of 203 gm. Na_2O. On taking the figures for Na_2O in the entire body of pregnant gilts, corresponding to the potash results quoted above (Missouri Bull. 114 (20)), the percentage of Na_2O in the entire body is found to be 0·14.

The balance work shows that in the normal group of sows a retention of 1·324 gm. Na_2O corresponds to a live weight increase of 663 gm. so that the percentage of Na_2O in the live weight increase becomes 0·20, a figure slightly higher than that obtained from analysis of the entire carcase.

The calcium-deficient group retained 1·764 gm. Na_2O per 663 gm. live weight increase which, expressed as a percentage, gives the figure 0·26, which is considerably higher than 0·14 per cent. arrived at from analytical data.

This abnormality, however, can be explained from the following summary:

	K_2O per cent. of entire body		Na_2O per cent. of entire body	
	Normal sows	Ca-def. sows	Normal sows	Ca-def. sows
Analytical data ...	0·20	—	0·14	—
Balance results ...	0·25	0·14	0·20	0·26

It is significant that the results of direct analysis and balance work should show such good agreement for both potash and soda in the normal group of sows. The low retention of potash by the calcium-deficient sows, however, is compensated by a correspondingly high conservation of soda. These results indicate that when lime is deficient in the food, the potash combines with the phosphoric acid and is excreted in the faeces, its function in the body for combining with proteins, etc., being replaced by sodium.

To summarise the above findings, the retentions of the various elements during the period of gestation were as follows:

			Normal group (gm.)	Ca-def. group (gm.)
Retention of	N	=	1439	1125
,,	CaO	=	736	21
,,	P_2O_5	=	720	647
,,	K_2O	=	201	111
,,	Na_2O	=	152	203

The addition of calcium carbonate to the ration resulted in an increased retention of nitrogen, calcium, phosphate and potash, but a decreased retention of soda.

The observation made in discussing the lime balances of the calcium-deficient sows, that rough lime equilibrium had been attained on a very small calcium intake, suggested that it would be interesting to determine the nitrogen and mineral balances of 32 p 3 immediately after weaning and also to find whether equilibrium was attained before the gilts were first bred. The three balances taken are recorded in Table X.

Table X. *Showing the nitrogen and mineral balances of two calcium-deficient gilts and also* 32 p 3 *immediately after weaning.*

		Output (gm.)				
	Intake	Faeces	Urine	Washings	Total output	Balance (gm.)
Gilt 64 S 7.						
Nitrogen ...	67·40	15·77	40·42	2·22	58·41	8·99
Calcium (CaO) ...	1·614	0·860	0·228	0·239	1·327	0·287
Phosph. acid (P_2O_5)	24·79	7·973	12·750	0·616	21·34	3·45
Potash (K_2O) ...	14·87	5·286	6·788	0·606	12·68	2·19
Soda (Na_2O) ...	9·73	1·894	5·687	0·514	8·09	1·64
Ash	71·73	27·85	28·34	2·79	58·98	12·75
Gilt 58 S 7.						
Nitrogen	59·35	16·00	32·09	2·81	50·90	8·45
Calcium (CaO) ...	1·365	0·941	0·140	0·060	1·141	0·224
Phosph. acid (P_2O_5)	21·700	9·57	9·14	0·660	19·37	2·33
Potash (K_2O) ...	14·40	5·43	6·75	0·814	12·99	1·41
Soda (Na_2O) ...	8·87	2·161	3·98	0·503	6·64	2·23
Ash	69·65	35·12	25·46	3·284	63·86	5·79
Sow 32 p 3 (*after weaning*).						
Nitrogen	69·93	17·16	44·80	1·76	63·72	6·21
Calcium (CaO) ...	2·076	1·371	0·232	0·118	1·721	0·355
Phosph. acid (P_2O_5)	24·53	10·44	11·82	0·392	22·65	1·88
Potash (K_2O) ...	15·35	6·94	7·81	0·442	15·20	0·15
Soda (Na_2O) ...	10·29	2·046	7·05	0·490	9·59	0·70
Ash	83·68	43·31	32·03	2·12	83·68	6·22

The nitrogen and mineral balances of both gilts are fairly consistent and the results indicate considerable nitrogen retention. Although the retention of phosphate was approximately eleven times that of the lime, it is noteworthy that both of these gilts show a small but consistent lime retention on a very small calcium intake. Of equal importance is the fact that 32 p 3 after weaning shows the same phenomenon so that the conclusion arrived at is that the lime balance is not enhanced when there is no foetal requirement. The amount of milk given by this sow during the lactation period prior to the balance determination can be judged from the fact that the live weight of the piglings at birth was 2·90 lb. but when weaned at seven weeks old the average weight was

only 9·62 lb. or one-third the weight of normal pigs of the same age. The young pigs made an average gain of less than one pound per week. The phosphate retention in this period was also low, suggesting that the excessive conservation during gestation had not become exhausted by the low milk yield. Since only one period was taken immediately after weaning, however, the figures must be taken as mere indications and no conclusions can be drawn. Unfortunately, since the sows only reared one litter each, and since no litters were obtained from the deficient gilts for reasons already given in the section dealing with their life-histories[1], it was impossible to get animals to confirm the above findings.

THE DEGREE OF INTERDEPENDENCE OBSERVED IN THE METABOLISM OF THE SEVERAL ELEMENTS.

Lime and phosphate.

The ratio of lime to phosphate in the food is considered by many investigators of mineral metabolism to be of the greatest importance. To secure optimum results it has been suggested that this ratio should be as nearly as possible identical with that of tricalcic phosphate, namely, 1·18, for then the degree of metabolic adjustment required for the deposition of calcium phosphate in the bones is said to be at its minimum. The above generalisation implies that the absorption of these two elements from the gut into the blood stream also takes place in the same ratio so that the lack of one would result in a diminished absorption of the other. The above conclusion, however, is in many cases based on short and often single mineral balance determinations where the imposition of an ill-balanced mineral diet has resulted in a physiological disturbance of metabolism and the utilisation of skeletal reserves. This has resulted in the negative balances recorded under these conditions being ascribed to the ratio of these two elements in the diet.

Whether the same results would be obtained if sufficient time were given for metabolic adjustment is debatable. That metabolic adjustment does take place is illustrated by the figures given in Table XI.

The ratios of lime to phosphate show that the metabolism of phosphate in the two groups must have been entirely different, but this does not prevent storage. That the utilisation of these two elements is not a simple matter of absorption in the ratio of tricalcic phosphate is clearly demonstrated by these figures. The results suggest that the animal can adjust itself to a fairly wide range of variation in lime and phosphate

[1] p. 762.

intake without suffering any appreciable harm. The fact that the calcium-deficient group of sows on a ration rich in phosphate attained lime equilibrium suggests the metabolism of these two elements to be to a

Table XI. *Showing the ratio of lime to phosphate ingested, excreted and retained by these two groups of sows.*

	Normal sows (gm.)	Ca-def. sows (gm.)
Average CaO-content of food ...	26·49	1·713
Average P_2O_5 in food	22·73	25·78
Ratio CaO/P_2O_5 in food ...	1·16	0·066
Average CaO in faeces	18·78	1·187
Average P_2O_5 in faeces	15·69	9·90
Ratio CaO/P_2O_5 in faeces ...	1·19	0·120
Average CaO in urine	1·086	0·216
Average P_2O_5 in urine	0·603	9·810
Ratio CaO/P_2O_5 in urine ...	1·80	0·022
Average CaO retained	6·40	0·183
Average P_2O_5 retained	6·26	5·62
Ratio CaO/P_2O_5 retained ...	1·02	0·032

considerable extent independent. Further proof is given by the relative amounts of lime and phosphate stored by the two groups at different periods. These were as follows:

	Normal group				Calcium-deficient group		
	Day of gestation	CaO retained (gm.)	P_2O_5 retained (gm.)		Day of gestation	CaO retained (gm.)	P_2O_5 retained (gm.)
8 N 2	13th	8·35	7·53	77 N 8	12th	+0·220	+ 2·05
	49th	5·67	3·90		52nd	−0·284	+10·71
	98th	2·97	3·83		85th	−0·239	+10·00
7 S 1	11th	3·48	4·75		87th	+0·372	+ 3·08
	39th	10·82	9·44		108th	+0·417	+ 5·91
	70th	8·65	7·77	32 p 3	14th	−0·146	+ 1·24
	90th	7·96	8·42		31st	−0·300	+11·78
10 S 2	56th	6·96	6·56		73rd	−0·092	+ 6·94
	98th	2·51	4·19		77th	+0·594	+ 2·06
					95th	+0·809	+ 2·47

It is noteworthy that in four out of the nine periods taken with the normal sows the phosphate retention was higher than that of the lime and it is also significant that three of these were recorded within a month of parturition when calcification of the foetus should be most active. Again, in the calcium-deficient sows there is no correlation between lime and phosphate retention.

SUMMARY.

(1) The physiological effects of calcium-deficiency on pregnant sows are briefly discussed. Results are given which demonstrate that a deficiency of lime in the food has no detrimental effect on the live weight of the young piglings at birth thus showing that up to parturition it is the mother organism that suffers and not the offspring.

(2) Storage of nitrogen takes place throughout pregnancy but the results indicate a considerably enhanced conservation within three weeks of parturition. The average daily retention of nitrogen by the high-calcium group of sows was 12·51 gm. and by the calcium-deficient group 9·78 gm. The normal sows therefore stored 1439 gm. N and the calcium-deficient 1125 gm. N during the gestation period. It is shown that storage of protein during pregnancy is greatly in excess of the foetal requirement, so that the mother organism, during gestation, adds on a reserve supply of protein in preparation for parturition and lactation.

(3) Ash ingredients were retained at all stages of gestation by both groups of sows. The addition of calcium carbonate to the food, however, resulted in an increased retention of ash. The percentage of the ash intake in the faeces was very similar in both groups, but the percentage of the intake in the urine was distinctly higher in the calcium-deficient sows indicating a more economical utilisation of ash by this group.

(4) On the high calcium diet the advancement of gestation was accompanied by a *decrease* in the amount of lime retained. The balance results for the calcium-deficient sows show that they have attained lime equilibrium on an intake of less than 2 gm. CaO per day. This shows that the animal is able to reduce the lime losses from her body to a minimal figure, thus devoting the lime from her own bones and tissues for the development of the foetus. Lime economy, on a low lime intake, is enhanced with the advancement of gestation, increased retention being observed towards parturition in both the calcium-deficient sows.

The average daily retention of the normal sows was 6·40 gm. CaO, which represents a storage of 736 gm. during gestation. It is calculated that this storage is nearly five times the amount deposited in the foetus. The calcium-deficient sows stored 21 gm. CaO for the purpose of foetal development. From chemical analysis of young piglings it is computed that the calcium-deficient sows had to sacrifice at least 100 gm. CaO from their own organism for the calcification of the foetus only. This explains the lack of milk by these sows when they farrowed.

(5) The retention of phosphate is as active in the first half of gestation as in the second half and there is no indication of increased conservation with the approach of parturition as was observed in the case of protein. The phosphate retention of the lime-deficient animals is not only positive in all cases, but in three periods is higher than any recorded for the normal sows. The generalisation often made that "Storage of phosphate is not possible unless lime is being stored as well," is not supported by this work.

When lime is abundant in the food, the excretion of phosphate takes place mainly through the wall of the intestine, but when lime is deficient there is a distinct increase in urinary phosphate. The results clearly show that phosphate absorption is in no way interfered with by a lack of calcium in the diet, at least 55 to 66 per cent. of the phosphate intake being absorbed into the blood by the calcium-deficient animals.

The normal group of sows show an average daily retention of 6·25 gm. P_2O_5 or a total retention of 720 gm. during gestation. The average daily balance of the calcium-deficient group was 5·62 gm. P_2O_5 representing a total conservation of 647 gm.

(6) The potash requirement is considerably smaller than that of lime and phosphate. Increased conservation was observed within a short period of parturition. In the high-calcium group of sows, from 45–67 per cent. of the potash intake was excreted in the urine, but when lime was deficient increased excretion of potash through the wall of the intestine was observed.

The normal group of sows retained a daily average of 1·745 gm. K_2O or a total conservation during pregnancy of 201 gm. The calcium-deficient group on a similar intake retained only 0·962 gm. K_2O per day which represents a total storage of 111 gm. during the period of gestation. This shows that when lime was deficient in the food the retention of potash was decreased nearly 50 per cent.

An examination of the blood of both groups of sows demonstrated a deficit of approximately 1,800,000 in the red-cell count of the deficient pigs as compared with the normal sows, indicating a definite though not serious anaemia. Slight lymphocytosis was also observed in the calcium-deficient animals.

(7) The highest conservation of soda in four sows occurred within a month of parturition. The average daily retention of soda by the normal sows was 1·324 gm. Na_2O, and during the whole period of gestation 152 gm. The calcium-deficient sows, on the other hand, had a daily retention of 1·764 gm. Na_2O or a total conservation of 203 gm.

(8) The addition of $CaCO_3$ to the ration resulted in an increased retention of nitrogen, lime phosphate and potash, but in a decreased retention of soda.

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APPENDIX.

Table I. *Showing the chemical composition of samples of barley meal (on basis of dry matter).*

	Sample no.								
Constituent	1	2	3	4	5	6	7	8	9
Crude protein ...	8·813	14·943	9·495	9·482	10·720	14·990	12·520	14·610	14·710
Ether extract ...	3·616	2·527	2·277	2·387	2·607	2·359	2·435	2·461	1·823
N-free extractives	80·552	72·759	79·260	78·440	77·810	75·600	76·150	74·590	75·257
Crude fibre ...	4·513	7·115	5·761	6·404	5·424	4·469	5·732	5·529	5·870
Ash	2·505	2·552	3·212	3·283	3·443	2·573	3·164	2·792	2·340
CaO	0·055	0·117	0·097	0·129	0·099	0·108	0·120	0·104	0·101
P_2O_5	1·021	1·031	1·086	1·030	1·121	0·880	1·024	0·943	0·736
K_2O	0·652	0·624	0·752	0·548	0·798	0·554	0·740	0·735	0·589
Na_2O	0·050	0·097	0·016	0·027	0·003	0·045	0·015	0·002	0·044
Nitrogen (N) ...	1·410	2·391	1·519	1·517	1·716	2·398	2·004	2·337	2·354

Table II. *Showing the chemical composition of samples of maize meal (dry matter).*

Constituent	Sample no. 1	2	3	4	5	6	7	8	9
Crude protein ...	11·463	11·375	11·400	10·062	10·300	10·520	10·300	11·530	11·190
Ether extract ...	6·531	7·968	5·503	5·745	5·653	5·585	5·558	5·671	5·550
N-free extractives	78·405	73·968	79·010	80·485	80·340	80·120	80·690	79·200	79·524
Crude fibre ...	1·721	3·880	2·306	1·955	2·058	2·135	1·780	1·942	2·026
Ash	1·880	2·814	1·777	1·755	1·651	1·634	1·680	1·651	1·710
CaO	0·018	0·025	0·016	0·032	0·019	0·016	0·014	0·019	0·013
P_2O_5	1·076	1·359	0·947	0·881	0·845	0·878	0·854	0·851	0·873
K_2O	0·522	0·713	0·522	0·503	0·485	0·490	0·447	0·474	0·492
Na_2O	0·029	0·067	0·070	0·007	0·033	0·026	0·012	0·00	0·011
Nitrogen (N) ...	1·834	1·820	1·823	1·610	1·649	1·684	1·648	1·846	1·790

Table III. *Showing the chemical composition of samples of bean meal (dry matter).*

Constituent	Sample no. 1	2	3	4	5	6
Crude protein ...	25·663	29·231	28·610	28·160	26·750	25·411
Ether extract ...	1·406	2·108	1·720	1·442	1·800	2·341
N-free extractives ...	59·426	55·386	62·743	59·140	57·980	65·131
Crude fibre	10·470	8·762	3·328	8·489	10·110	3·964
Ash	3·035	4·513	3·599	2·774	3·355	3·153
CaO	0·273	0·202	0·145	0·222	0·241	0·191
P_2O_5	1·056	1·713	1·012	0·728	0·825	0·803
K_2O	1·425	1·693	1·586	1·447	1·457	1·326
Na_2O...	0·040	0·022	0·052	0·039	0·074	0·031
Nitrogen (N) ...	4·106	4·677	4·577	4·505	4·282	4·064

Table IV. *Showing the chemical composition of samples of blood meal (dry matter).*

Constituent	Sample no. 1	2	3	4	5	6	7
Crude protein ...	93·250	94·950	95·812	94·500	95·960	94·430	94·880
Ether extract ...	—	0·921	—	0·190	0·422	0·700	0·126
N-free extractives	4·387	2·143	1·258	1·853	0·370	1·481	1·776
Crude fibre ...	—	—	—	—	—	—	—
Ash	2·363	1·986	2·912	3·457	3·245	3·388	3·228
CaO	0·056	0·066	0·060	0·067	0·108	0·045	0·041
P_2O_5	0·276	0·260	0·226	0·229	0·244	0·276	0·252
K_2O	0·330	0·333	0·403	0·355	0·374	0·398	0·344
Na_2O	1·053	1·069	1·264	1·203	1·036	1·082	1·187
Nitrogen (N) ...	14·920	15·190	15·330	15·120	15·360	15·110	15·180

Table V. *Showing the mean daily weight and composition of faeces (average of two composite samples). Normal group of sows.*

	Sow no. 8 N 2			Sow no. 7 S 1						Sow no. 10 S	
Period	1	2	3	Gilt 1	2	3	4	5	6	1	2
Fresh weight in gm....	1327	1322	1610	1174	1161	1599	1620	1350	1287	1270	1654
% dry matter ...	30·11	31·74	30·89	27·35	28·58	27·17	27·81	28·50	29·25	29·03	30·72
% N in fresh faeces	1·138	1·275	1·531	1·300	1·398	1·209	1·288	1·117	1·142	1·245	1·319
Weight of dry matter	398·8	419·9	497·4	321·8	331·7	434·4	450·6	384·05	376·98	368·85	507·95
Composition of faeces (dry matter):											
Crude protein ...	23·56	24·29	30·90	27·51	29·05	26·69	28·56	23·49	23·18	24·64	25·36
Ether extract ...	7·317	6·21	6·46	8·28	5·44	5·05	4·92	4·79	5·28	4·70	5·94
N-free extractives	38·32	36·58	34·02	34·93	36·49	37·56	36·36	40·42	40·08	37·90	38·97
Crude fibre ...	15·03	17·23	14·63	16·45	14·76	17·59	17·16	15·87	15·99	16·70	16·23
Ash	15·78	15·67	14·01	12·83	14·27	13·10	13·01	15·46	15·48	16·06	13·51
CaO	4·358	4·764	4·491	3·936	4·399	3·837	3·728	5·355	5·643	5·043	4·116
P_2O_5	4·144	4·290	3·614	4·107	3·775	3·326	3·164	4·008	3·988	4·266	3·250
K_2O	1·050	1·006	1·039	0·932	1·104	1·109	1·180	1·145	1·060	1·274	0·814
Na_2O	0·4211	0·4603	0·4479	0·5214	0·4639	0·4003	0·6791	0·5443	0·5253	0·4448	0·6220
N	3·770	3·889	4·944	4·410	4·649	4·269	4·569	3·758	3·708	3·934	4·057
CaO/P_2O_5	1·05	1·11	1·24	0·96	1·17	1·15	1·18	1·33	1·42	1·18	1·26

Table VI. *Showing the mean daily weights and composition of faeces (average of two composite samples). Calcium-deficient group of sows.*

	Sow no. 77 N 8					Sow no. 32 p 3						Gilt	Gilt
Period	1	2	3	4	5	1	2	3	4	5	6	64 S 7	58 S 7
Fresh wt. in gm. ...	1219	1563	1280	1522	1250	1390	1558	1949	1366	1479	1485	1323	1340
% dry matter ...	31·19	28·33	27·54	30·00	29·90	27·92	29·03	27·23	28·62	27·29	27·59	27·94	27·35
% N in fresh faeces	1·459	1·050	1·139	1·135	1·130	1·455	1·373	1·083	1·256	1·328	1·226	1·193	1·199
Wt. of dry matter	379·7	443·0	355·0	456·6	373·1	387·9	451·9	530·6	390·9	403·8	409·8	369·8	366·3
Composition of faeces (dry matter):													
Crude protein ...	27·35	22·86	22·99	22·76	22·76	31·90	30·14	23·31	26·17	28·37	26·84	25·00	25·78
Ether extract ...	6·56	8·49	7·02	5·81	7·83	7·93	6·88	7·91	7·46	7·65	8·00	7·43	8·37
N-free extractives	36·91	38·34	37·91	40·11	39·39	32·42	35·31	41·42	38·13	37·48	38·18	40·16	38·70
Crude fibre ...	19·61	20·31	21·94	20·55	19·15	18·97	18·98	19·13	17·16	17·35	18·32	19·88	17·56
Ash	9·57	9·17	10·15	10·77	10·87	8·78	8·69	8·24	11·08	9·17	8·66	7·53	9·59
CaO	0·327	0·316	0·317	0·251	0·294	0·3086	0·2272	0·2378	0·3505	0·2512	0·3354	0·233	0·257
P_2O_5	2·558	2·630	2·209	2·169	2·252	2·379	2·309	2·454	2·671	2·277	2·304	2·161	2·612
K_2O	1·761	1·799	1·848	1·952	1·869	1·878	1·672	1·801	1·776	1·685	1·667	1·433	1·484
Na_2O	0·3008	0·4320	0·5177	0·2670	0·4586	0·5483	0·6968	0·3633	0·5235	0·6343	0·5689	0·5102	0·5902
N	4·375	3·658	3·677	3·642	3·641	5·103	4·822	3·730	4·187	4·538	4·294	3·999	4·125

Table VII. *Showing the average daily balances of sow* 8 N 2 (*normal group*).

Period 1. Period taken on 13th day. Increase in weight in 15 days = 21 lb.

Constituent	Intake in gm.	Output in gm. Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N) ...	71·54	15·21	38·40	1·88	55·49	16·05
CaO	27·06	17·44	1·121	0·153	18·71	8·35
P_2O_5	24·49	16·56	0·269	0·129	16·96	7·53
K_2O	15·04	4·19	7·373	0·609	12·17	2·87
Na_2O...	9·67	1·676	5·605	0·450	7·73	1·94
Ash	121·87	63·05	30·27	2·25	95·57	26·30
Period 2. Period taken on 49th day. Increase in weight in 10 days = 6 lb.						
Nitrogen (N) ...	74·05	16·86	46·87	2·461	66·19	7·86
CaO	26·50	20·08	0·532	0·222	20·83	5·67
P_2O_5	25·15	18·04	2·804	0·402	21·25	3·90
K_2O	17·74	4·21	10·03	0·850	15·09	2·65
Na_2O...	9·80	1·95	5·84	0·469	8·26	1·54
Ash	128·91	65·95	31·57	3·99	101·51	27·40
Period 3. Period taken on 98th day. Not weighed.						
Nitrogen (N) ...	82·53	24·65	50·56	2·05	77·26	5·27
CaO	26·52	22·35	0·974	0·228	23·55	2·97
P_2O_5	22·34	17·96	0·422	0·124	18·51	3·83
K_2O	14·76	5·172	7·066	0·633	12·87	1·89
Na_2O...	10·387	2·227	6·383	0·470	9·08	1·31
Ash	117·60	69·71	29·28	0·297	99·29	18·31

Table VIII. *Showing the average daily balances of sow* 7 S 1 (*normal group*).

Period 1. Period taken when she was a gilt (on oestrus). Increase in weight in 13 days = 21 lb.

Constituent	Intake in gm.	Output in gm. Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N) ...	56·52	15·26	27·33	2·73	45·32	11·20
Lime (CaO)	19·86	12·68	0·512	0·500	13·69	6·17
P_2O_5	20·58	13·20	0·525	0·380	14·11	6·47
K_2O	12·45	2·86	5·530	1·200	9·59	2·86
Na_2O...	12·53	1·69	7·090	1·190	9·97	2·56
Ash	—	—	—	—	—	—
Period 2. Period taken on 39th day. Increase in weight in 12 days = 6 lb.						
Nitrogen (N) ...	82·99	16·13	51·200	3·090	70·42	12·57
CaO	26·54	14·62	0·747	0·350	15·72	10·82
P_2O_5	22·46	12·55	0·250	0·218	13·02	9·44
K_2O	14·83	3·66	9·018	0·930	13·60	1·23
Na_2O...	10·40	1·54	6·813	0·658	9·01	1·40
Ash	117·93	47·40	30·660	4·300	82·36	35·57
Period 3. Period taken on 70th day. Increase in weight in 10 days = 10 lb.						
Nitrogen (N) ...	84·00	19·31	47·400	3·950	70·66	13·34
CaO	26·38	16·62	0·669	0·441	17·73	8·65
P_2O_5	22·52	14·39	0·112	0·250	14·75	7·77
K_2O	16·67	4·81	9·365	1·155	15·33	1·34
Na_2O...	9·59	1·75	5·110	1·333	8·19	1·40
Ash	120·06	56·92	32·440	5·150	94·51	25·55

Table VIII (*contd*).

Period 4. Period taken on 90th day. Increase in weight in 10 days = 8 lb.

Constituent	Intake in gm.	Output in gm. Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N) ...	83·80	20·88	40·240	2·250	63·27	20·43
CaO	26·40	16·94	1·251	0·253	18·44	7·96
P_2O_5	22·90	14·26	0·090	0·133	14·48	8·42
K_2O	16·77	5·32	8·105	0·931	14·35	2·41
Na_2O...	9·61	3·06	4·354	0·570	7·98	1·63
Ash	120·96	57·12	27·440	3·567	88·13	32·83

Period 5. Period taken on 12th day (sow turned). Increase in weight in 11 days = 1·0 lb.

Constituent	Intake in gm.	Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N) ...	82·65	15·03	57·190	3·040	75·26	7·39
CaO	26·35	20·65	0·671	0·364	21·68	4·67
P_2O_5	20·77	15·47	1·138	0·272	16·88	3·89
K_2O	15·03	4·43	9·015	1·024	14·47	0·56
Na_2O...	10·30	2·09	7·930	0·790	10·81	0·49
Ash	115·98	59·46	33·780	4·119	97·36	18·62

Period 6. Period taken on 11th day. Not weighed.

Constituent	Intake in gm.	Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N) ...	82·82	14·70	55·63	2·150	72·48	10·34
CaO	26·31	21·37	1·235	0·227	22·83	3·48
P_2O_5	20·95	15·12	0·919	0·165	16·20	4·75
K_2O	15·198	4·03	9·449	0·782	14·26	0·94
Na_2O...	10·16	1·97	7·388	0·532	9·89	0·27
Ash	116·42	58·59	33·30	2·86	94·75	21·67

Table IX. *Showing the average daily balances of sow* 10 S 2 (*normal group*).

Period 1. Period taken on 56th day. Increase in weight in 10 days = 13 lb.

Constituent	Intake in gm.	Output in gm. Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N) ...	83·01	15·95	51·26	1·56	68·77	14·24
CaO	26·471	18·74	0·48	0·29	19·51	6·96
P_2O_5	22·965	15·85	0·46	0·10	16·41	6·55
K_2O	16·567	4·69	9·20	0·97	14·86	1·71
Na_2O...	9·668	1·62	6·45	0·53	8·60	1·07
Ash	120·73	59·47	26·45	2·67	88·59	32·14

Period 2. Period taken on 98th day. Increase in weight in 10 days = 10 lb.

Constituent	Intake in gm.	Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N) ...	82·68	21·75	46·88	1·55	70·18	12·50
CaO	26·304	20·85	2·772	0·166	23·79	2·51
P_2O_5	20·837	16·47	0·104	0·074	16·55	4·19
K_2O	15·123	4·122	9·819	0·507	14·448	0·675
Na_2O...	10·170	3·206	5·361	0·237	8·804	1·366
Ash	116·15	68·85	34·40	2·08	105·33	10·82

Table X. *Showing the average daily balances of sow* 77 N 8 (*calcium-deficient group*).

Period 1. Period taken on 52nd day. Increase in weight in 12 days = 13 lb.

Constituent	Intake in gm.	Output in gm. Faeces	Output in gm. Urine	Output in gm. Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N) ...	72·60	17·76	45·81	1·92	65·49	7·11
CaO	1·284	1·240	0·213	0·115	1·568	−0·284
P_2O_5	27·27	9·70	6·41	0·45	16·56	10·71
K_2O	16·46	6·70	7·30	0·78	14·78	1·68
Na_2O...	11·57	1·143	7·22	0·55	8·91	2·66
Ash	77·11	36·33	30·29	2·77	69·39	7·72

Period 2. Period taken on 85th day. Increase in weight in 11 days = 18 lb.

Constituent	Intake in gm.	Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N) ...	84·99	16·41	56·58	2·13	75·12	9·87
CaO	2·026	1·397	0·247	0·143	1·787	0·239
P_2O_5	31·321	11·655	9·130	0·531	21·32	10·00
K_2O	18·774	7·950	8·858	0·537	17·347	1·43
Na_2O...	12·604	1·910	7·348	0·631	9·889	2·72
Ash	91·39	40·53	35·52	2·99	79·04	12·35

Period 3. Period taken on 87th day. Not weighed.

Constituent	Intake in gm.	Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N) ...	71·63	14·76	47·18	2·41	64·34	7·29
CaO	1·783	1·125	0·184	0·102	1·411	0·372
P_2O_5	22·170	8·03	10·59	0·47	19·09	3·08
K_2O	15·27	6·572	7·262	0·891	14·73	0·54
Na_2O...	10·445	1·790	7·936	0·533	10·26	0·185
Ash	77·60	35·80	32·56	3·270	71·63	5·97

Period 4. Period taken on 107th day. Increase in weight in 9 days = 5 lb.

Constituent	Intake in gm.	Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N) ...	78·15	17·28	39·52	1·42	58·22	19·93
CaO	1·945	1·147	0·206	0·175	1·528	0·417
P_2O_5	24·18	9·902	8·102	0·265	18·269	5·91
K_2O	16·66	8·913	4·768	0·586	14·27	2·39
Na_2O...	11·395	1·219	5·147	0·535	6·901	4·494
Ash	84·65	49·20	22·96	3·167	75·33	9·32

Period 5. Period taken on 12th day. Increase in weight in 11 days = 13 lb.

Constituent	Intake in gm.	Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N) ...	83·91	14·28	61·66	1·87	77·81	6·10
CaO	1·667	1·103	0·211	0·133	1·447	0·220
P_2O_5	21·236	8·450	10·438	0·302	19·19	2·05
K_2O	15·389	6·984	7·040	0·738	14·76	0·63
Na_2O...	11·249	1·709	9·032	0·687	11·43	−0·18
Ash	75·40	39·77	30·09	3·15	73·01	2·39

Table XI. *Showing the average daily balances of sow* 32 p 3 (*calcium-deficient group*).

Period 1. Period taken on 31st day. Increase in weight in 10 days = 19 lb.

Constituent		Intake in gm.	Output in gm. Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N)	...	72·58	20·15	38·60	1·45	60·20	12·38
CaO ...	... ...	1·29	1·19	0·26	0·14	1·59	−0·30
P_2O_5 ...	... ...	27·17	9·18	5·82	0·38	15·38	11·78
K_2O ...	... ...	16·43	7·28	7·13	0·75	15·16	1·27
Na_2O...	... ...	11·20	2·13	6·75	0·65	9·53	1·67
Ash ...	... ...	76·17	34·06	29·58	2·73	66·37	9·79

Period 2. Period taken on 73rd day. Increase in weight in 10 days = 3 lb.

Constituent		Intake in gm.	Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N)	...	73·49	21·21	48·11	2·35	71·67	1·82
CaO ...	... ...	1·226	1·022	0·222	0·074	1·32	−0·09
P_2O_5 ...	... ...	27·91	10·41	9·96	0·595	20·97	6·94
K_2O ...	... ...	16·77	7·58	8·38	0·685	16·65	0·12
Na_2O...	... ...	11·22	3·15	6·66	0·513	10·32	0·90
Ash ...	... ...	78·51	39·58	34·60	2·720	76·90	1·61

Period 3. Period taken on 95th day. Increase in weight in 11 days = 23 lb.

Constituent		Intake in gm.	Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N)	...	85·39	21·11	52·46	2·51	76·08	9·31
CaO ...	... ...	2·338	1·263	0·180	0·086	1·529	0·809
P_2O_5 ...	... ...	31·44	13·030	15·340	0·598	28·97	2·47
K_2O ...	... ...	18·86	9·55	7·58	0·744	17·87	0·99
Na_2O...	... ...	12·76	1·92	6·79	0·336	9·04	3·72
Ash ...	... ...	91·80	43·71	31·83	2·290	77·83	13·97

Period 4. Period taken after weaning and oestrus. Increase in weight in 14 days = 14 lb.

Constituent		Intake in gm.	Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N)	...	69·93	17·16	44·80	1·76	63·72	6·21
CaO ...	... ...	2·076	1·371	0·232	0·118	1·721	0·355
P_2O_5 ...	... ...	24·53	10·44	11·82	0·392	22·65	1·88
K_2O ...	... ...	15·35	6·94	7·81	0·442	15·20	0·15
Na_2O...	... ...	10·29	2·046	7·05	0·490	9·59	0·70
Ash ...	... ...	83·68	43·31	32·03	2·120	83·68	6·22

Period 5. Period taken on 77th day. Loss in weight in 10 days = 3 lb.

Constituent		Intake in gm.	Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N)	...	78·25	19·63	47·03	2·51	69·17	9·08
CaO ...	... ...	1·920	1·013	0·153	0·160	1·326	0·594
P_2O_5 ...	... ...	23·95	9·181	12·175	0·532	21·89	2·06
K_2O ...	... ...	16·572	6·808	8·846	0·822	16·476	0·096
Na_2O...	... ...	11·366	2·561	7·285	0·588	10·434	0·932
Ash ...	... ...	83·77	37·03	34·95	3·69	75·67	8·10

Period 6. Period taken on 14th day. Increase in weight in 11 days = 15 lb.

Constituent		Intake in gm.	Faeces	Urine	Washings	Total output in gm.	Balance retained in gm.
Nitrogen (N)	...	83·80	18·20	56·92	1·69	76·81	6·99
CaO ...	... ...	1·655	1·373	0·294	0·134	1·801	−0·146
P_2O_5 ...	... ...	21·142	9·442	10·140	0·320	19·902	1·240
K_2O ...	... ...	15·300	6·836	7·362	0·618	14·816	0·484
Na_2O...	... ...	11·377	2·334	8·064	0·437	10·835	0·542
Ash ...	... ...	75·03	35·48	32·76	2·634	70·88	4·150

Table XII. *Giving a summary of the nitrogen and mineral balances.*

Experimental no. of sow	Day of gestation	Live weight increase per 10 days (lb.)	Balance in gm. of: Nitrogen	CaO	P_2O_5	K_2O	Na_2O	Ash
Normal sow 8 N 2	13th	14·0	16·05	8·35	7·53	2·87	1·94	26·30
	49th	6·0	7·86	5·67	3·90	2·65	1·54	27·40
	98th	*	5·27	2·97	3·83	1·89	1·31	18·31
Normal sow 7 S 1	†	0·90	7·39	4·67	3·89	0·562	−0·050	18·62
	11th	*	10·34	3·48	4·75	0·937	0·271	21·69
	39th	5·0	12·57	10·82	9·44	1·23	1·40	35·57
	70th	10·0	13·34	8·65	7·77	1·34	1·40	25·55
	90th	8·0	20·43	7·96	8·42	2·41	1·63	32·83
Normal sow 10 S 2	56th	13·0	14·24	6·96	6·55	1·71	1·07	32·14
	98th	10·0	12·50	2·52	4·19	0·675	1·366	10·82
Ca-def. sow 77 N 8	12th	11·8	6·10	0·220	2·05	0·627	−0·180	2·39
	52nd	10·8	7·11	−0·284	10·71	1·68	2·66	7·72
	85th	1·64	9·87	0·239	10·00	1·43	2·72	12·35
	87th	—	7·29	0·372	3·08	0·544	0·186	5·97
	107th	5·50	19·93	0·417	5·91	2·39	4·494	9·32
Ca-def. sow 32 p 3	‡	10·00	6·21	0·355	1·88	0·15	0·70	6·22
	14th	13·60	6·99	−0·146	1·24	0·484	0·542	4·15
	31st	19·0	12·38	−0·30	11·78	1·27	1·67	9·79
	73rd	3·0	1·82	−0·09	6·94	0·12	0·90	1·61
	77th	2·70	9·08	0·594	2·06	0·096	0·932	8·10
	95th	20·9	9·31	0·81	2·47	0·99	3·72	13·97
Balances of three gilts:								
Normal gilt 7 S 1	...	1·50	11·20	6·17	6·47	2·86	2·56	—
Ca-def. gilt 64 S 7	...	10·0	8·99	0·287	3·45	2·19	1·64	12·75
Ca-def. gilt 58 S 7	...	2·30	8·45	0·224	2·33	1·41	2·23	5·79

* Not weighed.
‡ After weaning.
† Sow turned after service.

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THE INFLUENCE OF THE ADDITION OF CALCIUM CARBONATE TO A RATION LOW IN LIME ON THE APPETITE AND DIGESTIBILITY OF THE FOOD IN SWINE.

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In the literature dealing with the effects of mineral deficiency it is often suggested that an animal on a deficient ration will, under these circumstances, consume more food in an endeavour to satisfy itself and make good the deficiency. This capacity to consume more food naturally depends, however, upon whether the appetite of the animal remains unimpaired. In this work on calcium deficiency in pregnant sows an opportunity was given of testing this point with particular reference to calcium and with animals that had been on a calcium-deficient diet for two generations.

It was observed that when the calcium-deficient sows were put in the metabolic crate for determining their mineral balances, much difficulty was experienced sometimes in getting them to clean up their troughs satisfactorily but no difficulty was experienced at any time with the normal sows. This was particularly the case with the former towards the end of gestation and in three instances the calcium-deficient sows had to be taken out of the crate, without taking a period, because they refused to eat any food. After two or three days, however, their appetite would return and then they were put back in the crate and consumed their food normally.

On going through the records for the whole experiment it was found that this was quite a consistent finding both towards the end of gestation and during lactation; the calcium-deficient sows periodically showing lack of appetite and refusing their food. Since no observation to this effect was recorded for the group receiving calcium carbonate, this "loss of appetite" must be attributed to a deficiency of lime.

An important point, therefore, arose as to whether an animal suffering from prolonged calcium deficiency is able to utilise the organic constituents of the food to the same extent as the normal animal or whether its digestive mechanism becomes impaired. Thus, the beneficial effect of the addition of calcium carbonate and the consequent balancing of the

lime-phosphate ratio might be attributed to some extent to a more perfect utilisation of the organic constituents. For the purpose of investigating this point eleven digestibility trials were taken with each of the two groups of sows. The average digestibility coefficients (mean of two sub-periods) are given in Table I.

Table I. *Digestion results per cent.*

Experimental no. of sow		Dry matter	Organic matter	Crude N	Ether extract	N-free extractives	Crude fibre	Ash	Day of gestation
Normal or high-calcium group.									
8 N 2	(1)	84·7	86·6	78·4	73·3	91·7	38·5	43·3	13th
	(2)	84·8	86·5	77·2	76·2	92·2	22·5	48·8	49th
	(3)	81·9	83·7	70·2	69·6	91·2	16·9	40·7	98th
7 S 1	(4)	86·5	88·1	82·2	79·9	92·3	40·2	49·7	11th
	(5)	86·1	87·8	81·8	81·5	92·0	38·5	47·1	12th
	(6)	88·0	89·3	80·9	83·0	93·8	44·5	59·8	39th
	(7)	84·2	85·6	77·0	79·6	91·4	21·5	52·6	70th
	(8)	83·6	85·1	75·1	78·1	91·5	22·5	51·5	90th
	(9)	84·7	85·7	73·1	74·2	92·4	16·8	62·1	Gilt
10 S 2	(10)	86·8	88·3	80·8	82·9	92·8	34·8	50·8	56th
	(11)	81·7	83·5	73·7	67·7	89·8	16·8	40·7	98th
Average	...	84·8	86·4	77·3	76·9	91·9	28·5	49·7	—
Calcium-deficient group.									
77 N 8	(1)	86·6	87·7	83·0	71·1	92·6	30·0	47·3	12th
	(2)	86·2	87·2	75·5	81·6	93·4	10·7	52·9	52nd
	(3)	83·8	84·8	80·7	72·7	87·9	36·8	55·7	85th
	(4)	86·0	87·0	80·2	74·9	92·6	17·0	54·1	87th
	(5)	83·4	84·7	77·9	75·5	90·7	7·1	41·9	107th
32 p 3	(6)	85·2	86·1	78·3	67·5	92·1	25·4	52·7	14th
	(7)	85·5	86·8	72·2	77·1	93·7	14·8	55·2	31st
	(8)	83·7	84·6	70·3	77·6	92·1	3·7	49·8	73rd
	(9)	85·2	86·1	74·9	71·1	92·3	29·2	55·8	77th
	(10)	80·7	81·7	75·3	69·7	88·6	29·0	51·8	95th
	(11)	85·3	86·5	76·6	72·1	92·3	34·2	48·3	Not preg.
Av. Ca-def. sows.		84·7	85·8	76·8	73·7	91·7	21·6	51·4	—
Av. normal sows		84·8	86·4	77·3	76·9	91·9	28·5	49·7	—

The digestion coefficients show that the ration fed in this experiment was highly digestible and very suitable for pregnant sows. Further, the close agreement shown by the average digestion coefficients of dry matter, organic matter, crude nitrogen, N-free extractives and ash clearly indicates that the addition of calcium carbonate had no effect on the absorption of these constituents, but, on the other hand, the utilisation of fat and fibre was slightly enhanced. Much significance, however, cannot be attached to the digestion coefficients of the two latter constituents, since in the first place little is known about the chemical nature of the ether extract of the faeces, while on the other hand the digestibility of the fibre shows considerable variation in the same animal at different periods.

It is interesting to note that the digestion coefficients were distinctly lower in all the periods taken within three weeks of parturition compared with the coefficients obtained in the first half of gestation. This has been commented upon by Ver Eeke[1], who suggests that "the increasing size of the uterus may prove a mechanical difficulty and impede intestinal activity."

The conclusion arrived at from Table I, however, is that the digestive mechanism of an animal suffering from the effects of protracted calcium deficiency is just as efficient as that of the healthy normal animal. This is important in that it demonstrates that lime deficiency does not impair the normal capacity of the animal for elaborating digestive ferments, and it appears that the digestive juices are normal in character.

The symptoms described in dealing with the life-histories of the calcium-deficient sows, such as lack of milk on farrowing, cannot, therefore, be ascribed to a deficient digestion of the organic constituents of the food.

SUMMARY.

(1) Animals on a calcium-deficient diet suffer periodically from loss of appetite though the ration is in every other respect satisfactory.

(2) The results of twenty-two digestion trials show no enhanced effect on the digestibility of the organic constituents of the food on adding calcium carbonate to a lime-deficient ration.

In conclusion, I wish to express my thanks to Prof. T. B. Wood, F.R.S., for giving me the necessary facilities to carry out the work; to Mr H. R. Davidson, M.A., for placing the experimental animals at my disposal and also to Dr H. E. Woodman for his active interest and supervision at all stages of the work.

[1] Marshall's *Physiol. of Reproduction*, p. 524.

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STUDIES IN THE GEOLOGY AND MINERALOGY OF SOILS.

II. SOILS OF SOUTH-EAST SCOTLAND.

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In the previous paper (1) the writer discussed in detail the geology and mineralogy of the soils of a small area within the south-east of Scotland. The soils were shown to contain varying amounts of silicate minerals in a fairly fresh state and the results suggested that a study of the mineralogy of the soils in the wider area might lead to a grouping of the soils on a petrographic basis. Such a petrographic study is also useful in indicating not only the genesis of the soil but also the state of weathering of the soil minerals. This has been emphasized recently in a memoir by Van Baren (2) on certain limestone soils from Java and Sumatra.

The area from which the soils were taken for this study lies between the Rivers Forth and Tweed and comprises the counties of West Lothian, Midlothian, East Lothian, Berwickshire, Roxburghshire, Selkirkshire and Peeblesshire.

Physiography.

This area forms the eastern part of two of the topographic divisions of Scotland, the Midland Valley and the Southern Uplands. Along the Forth and stretching east and south along the coast is a low-lying plain, sometimes stretching for several miles inland and at other times, especially in Berwickshire, being confined to a narrow coastal strip. From this plain there is a gentle slope to about 750 feet. This part is also variable in extent and is diversified in the west by the Bathgate and the Pentland Hills and in the east by the Garlton Hills. Beyond this come the Moorfoot and Lammermuir Hills, which here form the northern boundary of the Southern Uplands. These have a varying breadth of five to ten miles and an average height of about 1500 feet. They are succeeded by the valley of the Tweed, with a topographic character similar to that already described for the Forth but much narrower in extent.

Two distinct types of cultivation are to be found in the area: in the low ground arable, and in the higher, especially in the Moorfoots and Lammermuirs, pasture.

GEOLOGY.

Descriptions of the geology of this area may be had in the maps and memoirs published by the Geological Survey for Scotland (3, 4). The following rock groups occur:

Pleistocene and Recent	Peat Alluvia Raised beaches Glacial sands and gravels Boulder clay
Carboniferous	
Old Red Sandstone	Upper Lower
Silurian	Upper Lower (Ordovician)

The Silurian rocks consist mainly of shales, grits and greywackes, while the Old Red Sandstone is characterised by sandstones, marls, conglomerates and associated igneous rocks. All the Carboniferous Series up to the Coal Measures are represented, and of these the Calciferous Sandstone and the Carboniferous Limestone cover the largest area. There is also a large development of lavas and tuffs, especially of Lower Carboniferous age.

Overlying these rocks there is a coating of glacial drift, boulder clay and glacial sands and gravels, of varying thickness. It is deepest in the low-lying ground and thins to the hills. Round the Firth of Forth and on the sea-coast occur raised beaches, while alongside the principal rivers are stretches of alluvia. Peat is very extensively developed on the Moorfoot and Lammermuir Hills.

The physical features of the area are a reflection of the geology. In the northern part the hills consist of igneous rocks, while the Moorfoot and Lammermuir Hills are carved out of highly folded Silurian sediments. In all cases the rocks of the hills have been much more resistant to weathering than the softer sediments of Carboniferous and Old Red Sandstone age.

DESCRIPTION OF SOILS.

Climatically there are two divisions in the area, one of a rainfall under 30 in. and the other of a rainfall of between 30 and 40 in. The first area is confined to a narrow strip at the coast. This grouping has been used by Ogg (5) in his general classification of Scottish soils but no detailed subdivision on a climatic basis is yet possible. In the present study, soils overlying the various geological formations represented in the area were taken to determine the mineralogical differences present, if any, and also the state of weathering of the minerals.

Table I. *List of soils examined.*

Soil	Locality	Description of surface soil	Geology
		Group I	
1	Synton Mains, Selkirkshire	Buffy brown silty loam, stoney	Glacial drift over Upper Silurian
2	Blackcastle Hill, Selkirkshire	Buffy brown medium loam	,, ,, ,,
3	Synton Mill, Selkirkshire	Grey-brown loam, gravelly	,, ,, ,,
4	Easter Langlee, Berwickshire	Reddish brown loam, slightly gravelly	,, ,, ,,
5	Spottiswood, Berwickshire	Buffy brown gravelly loam	,, ,, ,,
6	Flass, Berwickshire	Olive brown loam, stoney	,, ,, ,,
		Group II	
7	East Mendick, Peeblesshire	Fuscous black peaty soil, gravelly	Glacial drift over Lower Old Red Sandstone
8	New Kaimhouse, Peeblesshire	Bister brown loam, clayey	,, ,, ,,
9	Temple Mains, East Lothian	Reddish brown clayey loam	Glacial drift over Upper Old Red Sandstone
10	Innerwick, East Lothian	Brown friable loam	,, ,, ,,
11	Bassendean, Berwickshire	Reddish brown clayey loam	,, ,, ,,
		Group III	
12	Pylafoot, Roxburghshire	Olive brown silty loam	Glacial drift over Basalt (Carboniferous)
13	Kersmains, Roxburghshire	Brown medium loam	,, ,, ,,
14	Boghall, Midlothian	Chocolate and brown loam	Glacial drift over Basalt (Old Red Sandstone)
15	Yellow Craigs, East Lothian	Olive brown loam	Glacial drift over Trachyte (Carboniferous)
16	Abbey Mains, East Lothian	Reddish brown loam	Glacial drift over Trachyte Tuff (Carboniferous)
17	Bankhead, West Lothian	Grey-brown medium loam	Glacial drift over Tuff (Carboniferous)
		Group IV	
18	Ugstonrigg, East Lothian	Buffy brown loam	Glacial drift over Calciferous Sandstone Series
19	Alderston, East Lothian	Olive brown medium loam	,, ,, ,,
20	Weird's Wood, East Lothian	Cinnamon brown loam, gravelly	,, ,, ,,
21	Moffat Wood, East Lothian	Dark grey brown loam	,, ,, ,,
22	Samuelston, East Lothian	Reddish brown loam	,, ,, ,,
23	Pyothall, West Lothian	Grey brown loam	,, ,, ,,
24	Swinton Mill, Berwickshire	Snuff brown gravelly loam	,, ,, ,,
25	Simprim, Berwickshire	Umber brown silty loam	,, ,, ,,
26	Leithholm, Berwickshire	Olive brown medium loam	,, ,, ,,
		Group V	
27	Salton, East Lothian	Chocolate brown loam	Glacial drift over Carboniferous Limestone Series
28	Oxwell Mains, East Lothian	Reddish brown loam	,, ,, ,,
29	Skatterraw, East Lothian	Reddish brown loam	,, ,, ,,
30	Pathhead, Midlothian	Friable brown loam	,, ,, ,,

In Table I is given a list of the soils examined and their localities, with a note on the underlying rock formation. All the soils, save No. 4, which is formed on glacial sands and gravels, are derived from boulder clay. The colour was determined on the air-dried soil by aid of Ridgeway's Colour Standards (6).

Mechanical analysis.

The mechanical analyses of soils from this region have been studied by Gracie (7) and Nos. *A*, *B*, *C*, Table II, are taken from his paper. A few representative analyses are given in Table II.

It will be seen that the clay content is fairly high but it is typical of soils of this area developed over glacial drift. All these soils are derived from boulder clay and naturally the clay content is higher than it would have been for such soils developed on glacial sands. The fine sand fraction in every case forms a fairly high proportion of the "fine earth" and, since this is the material used for the mineralogical analysis, it may be taken as typical of the "fine earth" fraction of the soil.

Table II.

	A	*B*	*C*	*D*	*E*	*F*	*G*
Fine gravel ...	13·40	7·49	5·47	—	—	—	—
Coarse sand ...	13·76	20·55	24·75	24·89	18·70	19·15	17·65
Fine sand ...	27·17	20·82	29·23	31·38	30·04	39·76	31·19
Silt	10·70	9·78	5·70	11·44	9·00	7·77	8·62
Fine silt ...	11·10	15·00	8·13	12·16	12·33	10·76	11·71
Clay	5·70	9·75	7·75	12·82	19·65	12·00	11·13
Moisture ...	4·54	4·09	4·09	2·43	2·77	2·59	10·81
Ignition loss ...	12·82	11·09	12·48	5·62	6·77	6·74	8·26
Total	99·19	98·57	97·60	100·74	99·26	98·77	99·37

Soil	Locality	Geology
A	Kidlaw, East Lothian	Glacial drift over Lower Silurian
B	Humbie, East Lothian	" " "
C	Innerwick, East Lothian	Glacial drift over Upper Old Red Sandstone
D	Boghall, Midlothian	Glacial drift over basalt (Lower Old Red Sandstone)
E	Moffat Wood, East Lothian	Glacial drift over Calciferous Sandstone Series
F	Bolton, East Lothian	" " "
G	Pathhead, Midlothian	Glacial drift over Carboniferous Limestone Series

Mineralogical analysis.

The methods used in the mineralogical analysis of the soils have been detailed in the previous paper (1, p. 98). As before the fine sand fraction of the mechanical analysis has been taken and the minerals forming it separated into three groups according to density. These groups are characterised by quartz, orthoclase felspar and ferromagnesian silicates respectively. The percentage weights of the groups for the soils are given in Table III. It should be noted that owing to the difficulty of separation

the percentage figure for the ferro-silicate group is generally more accurate than that for the orthoclase group. For comparative purposes also not only must the percentage figures be taken but also the mineral composition of the groups.

Table III. *Results of analyses of fine sand fractions, given as percentages.*

Soil	Orthoclase group	Quartz group	Ferro-magnesian silicate group
		Group I	
1	1·7	94·4	3·9
2	2·4	93·5	4·1
3	0·9	97·1	2·0
4	4·7	92·6	2·7
5	0·9	94·6	4·5
6	0·5	97·7	1·8
		Group II	
7	2·2	95·2	2·6
8	1·9	97·0	1·1
9	0·7	96·0	3·3
10	2·4	94·6	3·0
11	0·7	98·0	1·3
		Group III	
12	3·2	94·7	2·1
13	3·4	93·5	3·1
14	4·3	89·2	6·5
15	2·6	95·2	2·2
16	1·5	94·4	4·1
17	37·5	58·0	4·5
		Group IV	
18	1·6	96·5	1·9
19	1·2	93·3	5·5
20	0·4	97·6	2·0
21	2·8	94·9	2·3
22	1·6	95·1	3·3
23	1·7	92·4	5·9
24	1·1	96·1	2·8
25	1·6	95·3	3·1
26	1·4	94·3	4·3
		Group V	
27	2·1	96·0	1·9
28	2·2	95·1	2·7
29	3·0	90·3	6·7
30	1·9	97·0	1·1

From the above list in Table III it will be seen that there is a distinct variation in the ferro-silicate percentage of soils developed from similar geological material, but it is only to be expected that there are local concentrates in the drift deposits as in sediments. Also the varying textures of the glacial drifts affect the rate of percolation of atmospheric waters and thus affect the rate of solution of the minerals. Different degrees of cultivation may also have some effect. From these analyses the only broad generalisation is that the soils over igneous rocks have the

higher ferro-silicate percentage. There does not seem to be any differentiation between the soils over Old Red Sandstone sediments and over Carboniferous sediments, as had been found by Hendrick and Newlands in their study of soils from other parts of Scotland (8, p. 266), though on the whole the percentages for soils over the Carboniferous are higher. The high percentage for the Orthoclase group in No. 4 is due to the presence of rock fragments, while in the case of No. 17 the remarkably high figure is due to the mineraloid palagonite.

Table IV. *Mineral suite—other than quartz.*

	Group I						Group II					Group III				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Plagioclase... ...	3	1	2	2	1	3	1	2	1	3	3	1	1	1	1	4
Orthoclase and Microcline ...	4	6	4	5	5	4	7	3	4	2	1	2	3	5	3	2
Iron-oxides ...	1	3	3	3	3	1	2	1	2	1	2	3	2	2	2	1
Augite	5	5	—	4	4	—	4	4	3	6	5	4	4	3	4	3
Enstatite	—	—	—	—	—	13	—	—	13	—	11	6	10	—	—	—
Hypersthene ...	—	—	—	13	—	—	—	—	14	8	—	13	—	—	—	—
Hornblende ...	9	4	6	8	7	6	6	9	5	—	10	—	11	7	9	—
Biotite	6	8	5	6	6	7	—	11	6	4	8	5	9	4	5	7
Muscovite	7	9	7	9	8	8	—	12	7	—	—	—	—	—	6	6
Epidote	—	—	—	—	—	11	—	8	—	—	—	—	—	—	11	—
Apatite	—	—	—	—	—	—	—	—	12	—	9	7	5	9	—	—
Garnet	10	—	9	10	9	5	3	5	9	7	6	8	6	8	8	5
Zircon	11	10	10	11	11	10	8	6	8	5	7	9	7	6	10	9
Tourmaline ...	8	7	8	7	10	9	5	7	10	—	4	10	8	11	7	8
Rutile	12	11	—	12	12	—	—	—	11	—	12	11	—	12	—	—
Staurolite	—	—	—	—	—	12	7	10	—	—	—	—	—	—	—	—
Chlorite	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Palagonite ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rock fragments ...	2	2	1	1	2	2	—	—	—	—	—	12	—	10	—	10

	Group IV									Group V			
	18	19	20	21	22	23	24	25	26	27	28	29	30
Plagioclase... ...	1	1	1	2	1	3	2	3	1	2	2	3	6
Orthoclase and Microcline ...	3	4	3	3	4	4	3	1	3	4	3	4	4
Iron-oxides ...	2	2	2	1	2	1	1	2	2	1	1	1	1
Augite	4	3	4	4	3	2	4	4	4	3	4	2	2
Enstatite	—	8	—	—	—	—	12	12	7	10	—	12	—
Hypersthene ...	—	—	—	10	—	10	—	—	—	—	—	15	—
Hornblende ...	8	10	—	8	—	5	11	13	12	—	—	13	—
Biotite	6	6	5	—	—	7	5	5	5	6	7	5	5
Muscovite	7	7	6	—	—	8	6	6	6	9	—	6	—
Epidote	12	12	—	—	—	—	—	—	—	—	8	11	—
Apatite	—	—	—	—	—	12	10	9	11	11	—	14	—
Garnet	5	5	—	6	5	6	7	7	8	5	5	7	3
Zircon	10	9	8	5	7	11	8	8	9	7	6	8	7
Tourmaline ...	11	11	7	7	6	9	9	10	—	—	—	10	—
Rutile	9	—	—	9	8	13	—	11	10	8	—	9	—
Staurolite	—	13	—	—	9	—	—	—	—	—	—	—	—
Chlorite	—	—	—	—	10	—	13	—	—	—	—	—	—
Palagonite ...	—	—	—	—	—	—	—	—	—	—	—	—	—
Rock fragments ...	13	—	—	11	11	—	—	—	13	12	—	—	—

Table III should be compared with Table IV, in which lists of the minerals found are given. The frequency of the occurrence of the minerals is indicated by the numbers, 1 meaning most frequent, 2, 3, 4, etc., indicating decreasing frequency. The numbers are not relative for different soils but only for a particular soil.

Description of the minerals in the fine sand fraction of the soils.

Quartz is the most abundant mineral in all the soils. It occurs in angular grains, often clear but generally spotted with iron-oxide inclusions along cracks. Inclusions of fluid and rutile are also common.

Plagioclase is very common in all the soils but varies in amount. It is very frequent in the soils overlying the igneous rocks of Carboniferous age, especially the more basic types. It is practically always turbid, that is, showing signs of chemical decomposition: it is very infrequently fresh. The grains are generally quadrate in shape.

Orthoclase is also fairly common. It is always fresher than plagioclase but is generally slightly turbid. Like plagioclase it occurs in quadrate grains and does not show the irregular outline as in quartz. Microcline is fairly common in soils over the Old Red Sandstone and the Carboniferous areas, but is not nearly so common in the Silurian areas. It is always fresh.

Iron-oxides are generally the commonest minerals in the heavy residues. Limonite and ilmenite changed to leucoxene are the most frequent, but haematite, ilmenite and magnetite also occur, the latter being the commonest of the three. The limonite and haematite occur in earthy form.

Augite is present in all the soils, sometimes very fresh, at other times weathered. It occurs in greeny brown grains of rather large size, angular and prismatic in shape. The titaniferous variety is fairly common in certain of the soils from Carboniferous areas.

Enstatite occurs as rather small greyish prismatic grains. It is rather infrequent and is commonest in the soils from Carboniferous areas.

Hypersthene was noted in only seven of the soils and is most frequent in those over Carboniferous rocks. It is brownish, pleochroic and prismatic in habit.

Hornblende occurs in rather small angular and prismatic grains; green generally but brown varieties and also a peculiar cinnamon brown type occur. It is practically always fresh.

Biotite and *muscovite* are present in every soil. They both occur in

rounded plates, the biotite usually brown but sometimes green and the muscovite colourless. The brown biotites are generally bleached. Inclusions are common.

Epidote has only been occasionally met with in certain of the soils. It occurs in yellow-green grains, pleochroic, and generally ovoid in shape.

Apatite was only noted infrequently. It occurs as acicular needles and also as rounded grains.

Garnet has been noted in practically every soil examined. Brown, pinkish brown and colourless varieties are met with, the two latter often in association. The grains are usually angular or sub-angular and of large size, and occasionally show cleavage.

Zircon. Complete crystal forms to ovoid grains are present, the former being very infrequent. Varieties with prism and pyramid termination developed, the other end being broken, have been met with. The ovoid grains are commonest and are frequently dusky. Zoned varieties have also been noted.

Tourmaline. Brown, greenish and also blue varieties have been noted, but the latter are very infrequent. Prismatic forms, with end terminations, are fairly frequent but rounded forms are commonest. A feature of the tourmalines in the soils developed over Carboniferous rocks is the presence of both completely prismatic types and completely rounded types.

Rutile is not of very frequent occurrence in the soils. It is generally deep brown but the yellow-brown variety has also been noted. It is irregular in habit. No twins have been seen.

Staurolite has been found only in a few of the soils. It occurs in rather large rounded grains with a golden brown colour.

Chlorite has been found only in soils from the Carboniferous areas. It occurs as rounded green grains.

Palagonite. This mineraloid was noted in only one soil. It is brown in colour, with a specific gravity below 2·6 and refractive index of about 1·47. It contains inclusions, which have not been determined. The grains are irregular in shape and highly angular.

Rock fragments are especially common in the soils over Silurian rocks. They are micro-felsitic in character.

The soils in general contain a large proportion of ferromagnesian silicate minerals, which are comparatively fresh, that is, only slightly altered chemically, and having a large reserve of bases.

Minerals and plant food.

Potash, phosphate and lime are the important constituents of minerals to agriculture. Chemical tests can readily determine the presence of such substances in the soil but more important still is it to determine the availability of them for plant food-stuffs. Plummer (9), who has investigated the solubility of various potash-bearing minerals in carbonated water, found the following order of solubility: biotite, muscovite, orthoclase, microcline. In the soils examined, all these minerals have been found, though in varying amounts. The freshness, which is an indication of the chemical alteration, is least in biotite and greatest in microcline, which would support the above conclusion, though muscovite has always been found fresh. There would seem to be, therefore, an appreciable supply of potash in all the soils, but since orthoclase is in general a commoner mineral in the soils than the micas the potash may not be readily available.

The principal phosphate-bearing mineral found is apatite and this has been noted only in many of the soils over the Carboniferous area and infrequently in the others. Its presence and amount, however, are sometimes difficult to judge, since it is necessary in certain cases to clean the soil grains with dilute acid and apatite goes readily into solution.

Plagioclase, augite, hornblende, garnet, and epidote are the main lime-bearing minerals present. The two latter may be neglected since they are very resistant to weathering. Augite and hornblende have been found remarkably fresh, though the augite is generally slightly weathered. Plagioclase, however, is practically always turbid in every soil, that is, altered chemically. The soils contain a fair proportion of lime-bearing minerals.

Relation of soils to geology.

It has already been noted that the soils over Silurian rocks are peculiar in the large amount of rock fragments present in the fine sand fractions. These fragments are micro-felsitic in character and would seem to denote that the weathering processes have not gone so far in the soil material as in the case of the other soils. The slight metamorphism to which the Silurian rocks have been subjected may account for this. The paucity in orthoclase is also another feature of the mineralogy of these soils.

The soils over the Old Red Sandstone areas are marked generally by the angularity of the grains, though this is not always the case, and garnet is generally the distinguishing mineral, though it has not been

found so common in these soils as Hendrick and Newlands (8) found it in the north of Scotland. The soil from Innerwick (No. 10) is peculiar in that the minerals of the ferro-silicate group are mainly iron-oxides, limonite and haematite being the commonest. This soil has been very intensively cultivated for a long time and the mineral content may be a reflection of the aeration and continued application of artificial manures to the soils.

Soils of Groups III and IV are from Carboniferous areas but differ in that the first are developed over igneous rocks while the second are over sediments. The higher ferro-silicate content is generally sufficient to distinguish them but this is not invariably the case. In the first group, though the mineral suite is practically the same throughout, the presence or the abundance of a certain mineral may serve as a distinguishing characteristic. The Boghall soil (No. 14) has a similar mineral suite to the Roxburghshire soils (Nos. 12 and 13): these three soils being developed over basalts. Soils Nos. 15 and 16 have a large amount of orthoclase present: they are developed over trachyte and trachytic tuff respectively. Soil No. 17 is peculiar in the high percentage of palagonite it contains. The soil lies on a palagonite tuff, and is practically residual there, being only a thin skin of drift over the solid rock. In soil No. 21 from West Lothian there occurs a type of hornblende (cinnamon brown in colour) which is typical of the quartz-dolerites lying to the west. In the soils of this district also certain brown decomposition products are found, probably mineraloid in character, but it is not known from what mineral they arise.

The mineral suite of the soils from the Carboniferous areas is practically identical with that of Hendrick and Newlands (8, p. 204) from other Carboniferous areas, augite and hypersthene being the distinguishing minerals. In the soils so far examined the results would indicate that the soils of the Carboniferous Limestone areas may be distinguished from the Calciferous Sandstone areas by the more frequent occurrence of garnet in the former but an insufficient number of soils have been investigated to be positive on this point.

The above results would indicate a variation in the mineral content of the glacial drift, since all the soils are derived from drift material and that this mineral variation is determined by the geological formation underlying the drift. In the area examined, the general movement of the ice has been from west to east, so that in certain cases there is an overlap of material, as in the case of certain West Lothian soils, but generally the underlying rocks have the main influence in the petrographic

character of the drift. In the case of East Lothian, this variation has been noted by the Geological Survey in the Memoir (4, p. 171), in which comment is made on the difference in colour and texture of the boulder clay according to the underlying geological formation. Practically all the minerals of the soils are to be found in local rocks and extraneous minerals, such as staurolite and tourmaline, which may have been derived from erratics, are infrequent and there is also the possibility that they may have been derived from local sediments since they have been recorded in sandstones of the Midland Valley of Bosworth (10).

Mineralogy of the soils and classification.

In the previous section it was shown that the soils had different mineral content according to the different geological formation underlying the boulder clay from which the soils were derived. Thus, in the south-east of Scotland the soils can be related petrographically to the underlying rocks through the drift. This is, of course, not expected to hold always, especially if the outcrops of rocks at right angles to the direction of ice movement are narrow and the drift thick. In regions of complex geology also the drift may be expected to be very varied. But where, as in south-east Scotland, extensive areas of similar rock formation occur the mineral constitution of the drift approximates to that of the underlying rock. A determination of the mineralogy of the matrix of the drift is in such cases useful in determining, first, the distribution of the drift, and, second, in forming a basis for grouping the soils.

Summary.

(1) The mineralogical composition of the fine sand fraction of certain soils from the south-east of Scotland is described.

(2) The soils are shown to possess a fairly high content of silicate minerals in a comparatively fresh state.

(3) The distribution and amount of potash, phosphate and lime-bearing minerals in the soils is discussed.

(4) The soils can be grouped according to their mineral content and this grouping is found to depend on the geology of the parent material.

(5) All the soils are formed on glacial drift and the results suggest that the local rocks have a preponderating influence on the composition of the matrix of the drift.

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